(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 September 2002 (26.09.2002)

PCT

(10) International Publication Number
WO 02/074305 A1

(51) International Patent Classification[7]: A61K 31/285, A61P 19/02

(21) International Application Number: PCT/AU02/00310

(22) International Filing Date: 19 March 2002 (19.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PR 3798 19 March 2001 (19.03.2001) AU

(71) Applicant *(for all designated States except US)*: **UNISEARCH LIMITED** [AU/AU]; University of New South Wales, Rupert Myers Building, Gate 14 Barker Street, Kensington, NSW 2052 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HOGG, Philip, John** [AU/AU]; 2/4-6 Clyde Street, Randwick, NSW 2031 (AU). **DONOGHUE, Neil** [AU/AU]; 7/26 Addison Street, Kensington, NSW 2033 (AU).

(74) Agent: **SPRUSON & FERGUSON**; G.P.O. Box 3898, Sydney, NSW 2001 (AU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/074305 A1

(54) Title: USE OF A SUBSTANTIALLY CELL MEMBRANE IMPERMEABLE COMPOUND FOR TREATING ARTHRITIS

(57) Abstract: The present invention provides a method of treatment and/or prophylaxis of arthritis in a vertebrate comprising administering to said vertebrate in need of said treatment and/or prophylaxis a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient, wherein said compound of formula (I) is defined as: $A-(L-Y)_p$, wherein: A comprises at least one substantially cell-membrane impermeable pendant group; L comprises any suitable linker and/or spacer group; Y comprises at least one arsenoxide or arsenoxide equivalent; p is an integer from 1 to 10; and the sum total of carbon atoms in A and L together, is greater than 6.

Use of a Substantially Cell Membrane Impermeable Compound for Treating Arthritis

Technical Field

The present invention relates to methods of treatment of arthritis.

Background of the Invention

Arthritis is a chronic multifactorial disease induced when the immune system attacks and begins degrading the body's joints. The disease comes in many forms, including calcific periarthritis, enteropathic arthritis, chronic arthritis, gout, hand arthritis, osteoarthritis, hip and knee osteoarthritis, thumb, Jaccoud's, and juvenile osteoarthritis, oligoarthritis, polyarthritis, and peripheral, psoriatic, rheumatoid, and septic arthritis. Rheumatoid arthritis alone is estimated to affect 1% of the world's population and is twice as prevalent in women as in men.

Reducing inflammation is the most commonly used treatment option in arthritis. The glucocorticosteroids, such as prednisolone and methylprednisolone, are often-used anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs (NSAIDs) are also used to suppress inflammation. NSAIDs inhibit the cyclooxygenase (COX) enzymes, COX-1 and COX-2, which are central to the production of prostaglandins produced in excess at sites of inflammation. In addition, the inflammation-promoting cytokine, tumor necrosis factor α (TNFα), is associated with multiple inflammatory events, including arthritis, and first generation anti-TNFα theraples are being used clinically.

There is a need however, for the development of therapeutically active drugs that block angiogenesis in the synovial tissue. These drugs should block leucocyte ingress that triggers inflammation.

Several angiogenesis modulating agents, such as TNP-470, taxol, thalidomide, and 2-methoxyestradiol, have been tried in rodent models of arthritis with varying success. In the present invention, a substantially cell membrane impermeable compound has been shown to be a very effective treatment of arthritis. Furthermore, there were no signs or symptoms of toxicity associated with the substantially cell membrane impermeable compound.

Therefore, the present invention provides a method of treatment of arthritis using substantially cell membrane impermeable compounds.

Disclosure of the Invention

According to a first embodiment of the invention, there is provided a method of treatment and/or prophylaxis of arthritis in a vertebrate comprising administering to said vertebrate in need of said treatment and/or prophylaxis a therapeutically effective amount of a compound of Formula (I)

or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient, wherein said compound of formula I is defined as

$$A\text{-}(L\text{-}Y)_p \qquad (I)$$

wherein

A comprises at least one substantially cell-membrane impermeable pendant group;

L comprises any suitable linker and/or spacer group;

Y comprises at least one arsenoxide or arsenoxide equivalent;

p is an integer from 1 to 10; and

the sum total of carbon atoms in A and L together, is greater than 6.

The following features relate to the compound of formula (I):

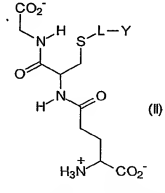
Typically, A is hydrophilic. More typically, A may be charged, uncharged or neutral at physiological pH.

Typically, A is selected from the group consisting of natural, unnatural and synthetic amino acids, hydrophilic amines, peptides, polypeptides, oligosaccharides, detectable groups, thiol containing proteins, or a combination thereof. More typically, A is selected from the group consisting of glutathione, glucosamine, cysteinylglycine, cysteic acid, aspartic acid, glutamic acid, lysine, arginine, wherein the sulfur atom of each sulfur containing compound may be optionally oxidised to form a sulfoxide or sulfone.

Amino acid side chains are known to those of skill in the art and are listed, for instance in standard reference texts, such as King and Stansfield, A Dictionary of Genetics, 4th Edition, Oxford University Press, 1990, the contents of which are incorporated herein by reference.

Still typically, pendant group A is a detectable group, such as biotin, cy™5.5 or fluorescein.

Even more typically, A is glutathione and in one form the compound of Formula (I) is represented by the following Formula (II):



(II)

wherein L comprises any suitable linker and/or spacer group and wherein Y comprises an arsenoxide or an arsenoxide equivalent.

Typically, Y is an arsenoxide group, and can be represented by –As=O.

Typically, p is an integer from 1 to 8. More typically, p is an integer from 1 to 5. Even more typically p is an integer from 1 to 3. Yet still more typically, p is 1.

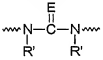
Typically, L corresponds to $(XBX')_nB'$. Typically, n is an integer from 0 to 20, more typically 0 to 15, even more typically 0 to 10, still more typically 0 to 5.

Still in accordance with compounds of Formula (I), the following relates to $(XBX')_nB'$.

Typically, X is selected from the group consisting of –NR, -S(O)-, -S(O)O-, -S(O)$_2$-, -S(O)$_2$O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R$_1$)-, and -P(O)(R$_1$)O-, or is absent;

B is selected from the group consisting of C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene and C_2-C_{10} acyl;

X' is selected from the group consisting of -NR-, –O-, -S-, -Se-, -S-S-, S(O)-, -OS(O)-, OS(O)O-, -OS(O)$_2$, -OS(O)$_2$O-, -S(O)O-, -S(O)$_2$-, -S(O)$_2$O-, -OP(O)(R$_1$)-, -OP(O)(R$_1$)O-, -OP(O)(R$_1$)OP(O)(R$_1$)O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R$_1$)-, -P(O)(R$_1$)O-, and



or is absent; wherein E is O, S, Se, NR or N(R)$_2$$^+$;

n is 0, 1 or 2; and

B' is selected from the group consisting of C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, and heteroarylene or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, OR$_2$ and C_2-C_{10} acyl;

R' is the same as R or two R' may be taken together with the nitrogen atoms to which they are attached to form a 5 or 6-membered saturated or unsaturated heterocyclic ring;

each R_1 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR$_2$ and N(R)$_2$;

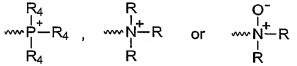
each R_2 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl and -C(O)R$_5$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10}

heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, OH, SH and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent) may be in a para-, meta- or ortho- relationship; and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, heterocycloalkylene, heterocycloalkenylene, arylene, heteroarylene and acyl may be independently substituted with hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-OS(O)R_3$, $-S(O)_2R_3$, $-OS(O)_2R_3$, $-P(O)R_4R_4$, $-OP(O)R_4R_4$, $-N(R'')_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$;



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$, $N(R)_2$ and $-C(O)R_5$;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, halo and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10}

cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R" is the same as R or two R" taken together with the N atom to which they are attached may form a saturated, unsaturated or aromatic heterocyclic ring system;

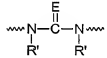
Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

More typically, X is selected from the group consisting of -C(O)-, -C(S)-, -C(O)O-, C(S)O-, and -C(S)S-, or is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -S-S-, -S(O)-, $-S(O)_2$-, $-P(O)(R_1)$-, $-OP(O)(R_1)$-, $OP(O)(R_1)O$-, $-OP(O)(R_1)OP(O)(R_1)O$-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -Se-,



, or is absent; wherein E is O, S or $N(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_5 selected from the group consisting of alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, and C_6-C_{12} arylene, or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, OR_2 and C_2-C_{10} acyl;

R' is the same as R;

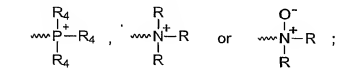
each R_1 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_2 and $N(R)_2$;

each R_2 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, and $-C(O)R_5$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH, and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, cyano, halo, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -S(O)$_2R_3$, -OS(O)$_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -N(R")$_2$, $NRC(O)(CH_2)_mQ$, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, -S(O)R_3, -S(O)$_2R_3$, -P(O)$(R_4)_2$, N$(R)_2$ and -C(O)R_5;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio and N$(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_5 cycloalkylthio, C_5-C_5 cycloalkenylthio, C_6-C_{12} arylthio, halo and N$(R)_2$;

R_6 is independently selected from the group consisting of C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, -S(O)R_3, -S(O)$_2R_3$ and –C(O)R_5,

R" is the same as R;

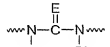
Q is selected from the group consisting of halogen and –OS(O)$_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Even more typically, X is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -S-S-, -S(O)-, -S(O)$_2$-, -P(O)(R_1)-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -Se-, and



, or absent; wherein E is O, S or $N(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and wherein

each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, OR_2 and C_2-C_5 acyl;

R' is the same as R;

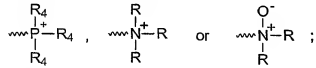
each R_1 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, halo, OR_2 and $N(R)_2$;

each R_2 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl and -C(O)R_5;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH and $N(R)_2$;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent) may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -S(O)$_2R_3$, -OS(O)$_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, $-N(R'')_2$, -NRC(O)$(CH_2)_mQ$, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, -S(O)R_3, -S(O)$_2R_3$, -P(O)$(R_4)_2$ and –C(O)R_5;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio and $N(R)_2$;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, halo and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R" is the same as R;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Still more typically, X is absent;

B is selected from the group consisting of C_1-C_5 alkylene, C_6-C_{12} arylene and C_2-C_5 acyl;

X' is selected from the group consisting of –O-, -S-, -NR-, -C(O)-, and -C(O)O-, or is absent;

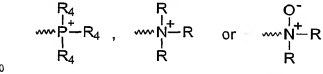
n is 1; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and

R is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl and C_2-C_5 acyl;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para-, meta- or ortho- relationship, and

wherein each alkylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)R_4R_4$, $-N(R")_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein each R is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl and C_2-C_5 acyl;

R_{2a} is selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$ and $-C(O)R_5$;

each R_3 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, and C_6-C_{12} arylthio;

each R_4 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, halo and $N(R)_2$;

each R_5 is independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, OH, SH and $N(R)_2$;

R_6 is selected from the group consisting of C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ and $-C(O)R_5$,

R" is the same as R above;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

Yet still more typically, X is absent;

B is C_2-C_5 acyl;

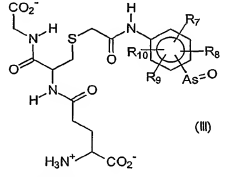
X' is NR;

n is 1;

B' is phenylene; and

R is H;

wherein the substituents directly attached to the phenylene ring may be in a para-, meta- or ortho-relationship, as exemplified by Formula (III):



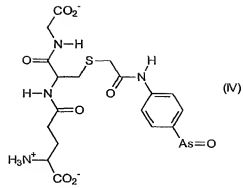
(III)

wherein R_7 to R_{10} are independently selected from the group consisting of hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, $-OS(O)_2R_3$ and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ and $-OS(O)_2$-*p* tolyl; and wherein, when any one of R_7 to R_{10} is C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, $-OS(O)_2R_3$ it is capable of forming a fused ring with the phenylene; and further wherein, at least one of R_7 to R_{10} is C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, or $-OS(O)_2R_3$, in combination with at least any one other of R_7 to R_{10}, is capable of forming a fused ring with the phenylene.

More typically, R_7 to R_{10} are independently selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, cyano, carboxy, C_1-C_5 alkoxy, methyl, ethyl, isopropyl, tert-butyl, phenyl and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ and $-OS(O)_2$-*p* tolyl.

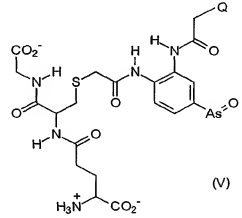
Further, when B' is arylene, the substituents attached to the arylene ring are typically in an ortho-, meta- or para- relationship to the –As=O. More typically the substituents are in a meta- or para- relationship to the –As=O group.

More preferably the compound of Formula (I) is 4-(*N*-(*S*-glutathionylacetyl)amino)-phenylarsenoxide, which can be abbreviated to GSAO, according to Formula (IV):



(IV)

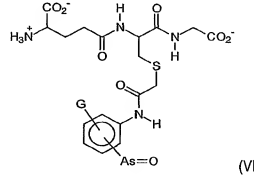
In a typical form, compounds of formula (I) are exemplified by compounds corresponding to Formula (V):



(V)

wherein Q is any halogen. For example, the invention provides the compounds 3-(*N*-(fluoroacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSFAO, 3-(*N*-(chloroacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSCAO, 3-(*N*-(bromoacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)-phenylarsenoxide, which may be abbreviated to GSBAO, and 3-(*N*-(iodoacetyl)amino)-4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide, which may be abbreviated to GSIAO.

In another preferred form, compounds of formula (I) are exemplified by compounds corresponding to Formula (VI):



(VI)

wherein G is selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, cyano, carboxy, C_1-C_5 alkoxy, C_1-C_5 alkyl and C_6-C_{12} aryl and -NHC(O)CH_2Q, wherein Q is halogen, -OS(O)$_2CH_3$, -OS(O)$_2C_6H_5$ or –OS(O)$_2$-*p* tolyl.

Typically, G is selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, methyl, ethyl, iso-propyl, tert-butyl, phenyl, and -NHC(O)CH_2Q, wherein Q is the group consisting of halogen, -OS(O)$_2CH_3$, -OS(O)$_2C_6H_5$ and –OS(O)$_2$-*p* tolyl.

More typically, in a compound of Formula VI, G is hydroxy, fluorine, amino, or nitro.

Typically, group G is in an ortho-, meta- or para- relationship to the arsenoxide group, more typically an ortho- or para- relationship.

Typically the activity of the arsenic atom may be modified by the group G, when G and the arsenic atom are in an ortho or para relationship to one another. For example, when G is an electron donating group such as OH (ionised to O^- at physiological pH), the arsenic atom should be deactivated towards dithiols and so become more selective, only reacting with very reactive dithiols. Alternatively, when G is an electron withdrawing group, such as NO_2, electron density would be drawn away from the arsenic atom, making it more reactive to all dithiols. Selective inhibition of some redox proteins and not others may be achieved by manipulation of G.

Still typically, compounds corresponding to Formula (I) may have the arsenoxide group (-As=O) replaced by an arsenoxide equivalent.

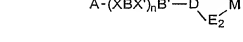
An arsenoxide equivalent is any dithiol reactive species that shows essentially the same affinity towards dithiols as –As=O. Typically, arsenoxide equivalent includes dithiol reactive entities, such as As, Ge, Sn and Sb species. More typically an arsenoxide equivalent can be represented by -D(Z_1)(Z_2). Arsenoxide equivalents are expected to exhibit identical or substantially identical activity to that of the corresponding arsenoxide.

Typically, for arsenoxide equivalents of the form -D(Z_1)(Z_2), D will be, for example, As, RSn, Sb, or RGe, and Z_1 and Z_2 will be labile groups (i.e. groups easily displaced under physiological conditions). Z_1 and Z_2, may be identical or different, and may either be connected or independent from each other (bound only to the arsenic atom).

Suitable arsenoxide equivalents include the following:

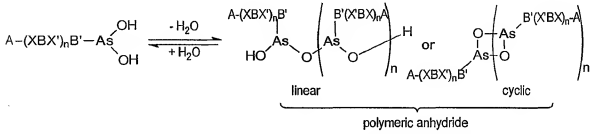
-D(Z_1)(Z_2),

wherein Z_1 and Z_2 are selected from the group consisting of OH, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, C_1-C_{10} alkylthio, C_6-C_{10} arylthio, C_1-C_{10} alkylseleno, C_6-C_{10} arylseleno, F, Cl, Br and I;

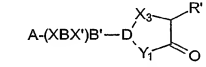


wherein $E_1 = E_2 = O$, $E_1 = O$ and $E_2 = S$ or $E_1 = E_2 = S$; M is R^m and R^n are independently selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, hydroxy and carboxy; and n = 1 to 10.

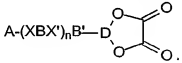
For arsenoxide equivalents of the form $D(Z_1)(Z_2)$, when D is As and Z_1 and Z_2 are OH, the arsenoxide equivalent may be in equilibrium with polymeric species, as depicted below.

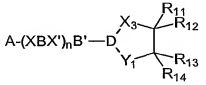


In respect of the equilibrium depicted above, arsenic is one of many elements whose hydroxy species exist in equilibrium with the corresponding polymeric anhydrides (Doak & Freedman, 1970). Therefore, arsenoxide compounds may actually exist as low or medium molecular weight polymers (eg n = 3 to 6). However, the dehydration reaction is reversible, and therefore soluble polymeric anhydrides are expected to behave as arsenoxide equivalents, that is, they are expected to bind to closely spaced dithiols in substantially the same way as the monomeric $-As(OH)_2$ species.

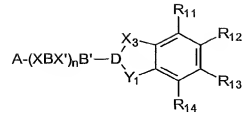


wherein X_3=NH, Y_1=O; X_3=Y_1=O or X_3=S, Y_1=O, and R' is selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, and carboxy, or is one of the twenty amino acid side chains;





wherein X_3=Y_1=O; X_3=NH, Y_1=O; X_3=S, Y_1=O; X_3=Y_1=NH; or X_3=S, Y_1=NH; and R_{11} to R_{14} are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, and CO_2H;



wherein X_3=Y_1=O, or X_3=NH, Y_1=O; and R_{11} to R_{14} are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_6-C_{12} aryl, halogen, C_1-C_{10} alkoxy, and CO_2H.

Yet still typically, compounds of formula (I) may be linked to detector groups.

Typically, the detector group may be a chemical group, for example, biotin, fluorescein, cy™5.5 or a group comprising a transition element.

Alternatively, the detector group is a radionucleide, such as ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{125}I, ^{131}I, ^{123}I, ^{111}In, ^{105}Rh, ^{153}Sm, ^{67}Cu, ^{67}Ga, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, and ^{99m}Tc.

More typically, the radionucleide detector group is ^{3}H or ^{14}C.

According to a second embodiment of the invention, there is provided a compound of Formula (I) as defined in the first embodiment of the invention, or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient, when used in the treatment and/or prophylaxis arthritis in a vertebrate in need of said treatment and/or prophylaxis.

According to a third embodiment of the invention, there is provided the use of a compound of Formula (I) as defined in the first embodiment of the invention, or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment and/or prophylaxis of arthritis in a vertebrate in need of said treatment and/or prophylaxis.

Arthritis comes in many forms, and typically includes calcific periarthritis, enteropathic arthritis, chronic, gout, and hand osteoarthritis, hip and knee osteoarthritis, thumb, Jaccoud's, and juvenile osteoarthritis, oligoarthritis, polyarthritis, and peripheral, psoriatic, rheumatoid, and septic arthritis.

Definitions

In the context of this specification, the term "comprising" means "including principally, but not necessarily solely". Furthermore, variations of the word "comprising", such as "comprise" and "comprises", have correspondingly varied meanings.

In the context of this specification, the term "arsenoxide" refers to the group –As=O.

In the context of this specification, the groups written –As=O and $–As(OH)_2$ are to be considered synonymous.

In the context of this specification, the term "arsenoxide equivalent" refers to any dithiol reactive species that shows essentially the same affinity towards dithiols as –As=O or $As(OH)_2$, and the term includes, for example, groups comprising a transition element, and any trivalent arsenical that is either hydrolysed to –As=O or $–As(OH)_2$ when dissolved in an aqueous medium (such as cell culture buffers and the fluids contained in the organism being treated).

The term "arsenical" as used herein, includes any compound that contains arsenic.

The term "acyl" as used herein, includes monovalent and divalent alkyl, alkenyl, alkynyl, cycloalkyl and cycloalkenyl moieties possessing a terminal carbonyl substituent wherein attachment may occur at the hydrocarbon moiety, the carbonyl moiety or both.

The term "alkyl" as used herein, includes within its meaning monovalent, saturated, straight and branched chain hydrocarbon radicals.

The term "alkenyl" as used herein, includes within its meaning, monovalent, straight and branched chain hydrocarbon radicals having at least one double bond.

The term "alkynyl" as used herein, includes within its meaning, monovalent, straight and branched chain hydrocarbon radicals having at least one triple bond.

The term "alkylene" as used herein, includes within its meaning divalent, saturated, straight chain hydrocarbon radicals.

The term "alkenylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one double bond.

The term "alkynylene" as used herein, includes within its meaning, divalent, straight chain hydrocarbon radicals having at least one triple bond.

The term "aryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "arylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

The term "closely spaced dithiol" as used herein, includes within its meaning thiols that are chemically vicinal, as well as thiols brought into spatial apposition by virtue of molecular conformation.

The term "cycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals.

The term "cycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "cycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having at least one double bond.

The term "halo" as used herein, includes fluoro, chloro, bromo and iodo.

The term "heteroaryl" as used herein, includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "heteroarylene" as used herein, includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic radicals having 1 to 12 atoms wherein 1 to 6 atoms are heteroatoms selected from O, N and S.

The term "heterocycloalkyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenyl" as used herein, includes within its meaning monovalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least 1 double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "heterocycloalkenylene" as used herein, includes within its meaning divalent, saturated, monocyclic, bicyclic, polycyclic or fused polycyclic radicals having at least one double bond and wherein 1 to 5 atoms are heteroatoms selected from O, N or S.

The term "phenylarsonic acid" as used herein, is to be considered synonymous with "benzene sulfonic acid".

The term "therapeutically effective amount" as used herein, includes within its meaning a non-toxic but sufficient amount a compound or composition of the invention to provide the desired therapeutic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

The term "transition element" as used herein, includes within its meaning the groups of elements comprising the transition metals, the lanthanides and the actinides.

Abbreviations

pAA, *p*-arsanilic acid, 4-aminobenzenearsonic acid; AspAO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-aspartic acid; BAE, bovine aortic endothelial; BCE, bovine capillary endothelial; BCS, bovine calf serum; BSA, bovine serum albumin; BRAA, 4-(*N*-(bromoacetyl)amino)phenylarsonic acid; BRAO, 4-(*N*-(bromoacetyl)amino)-phenylarsenoxide; BVS, bovine vascular smooth muscle; CAM, chick chorioallantoic membrane; Cys*AO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-cysteic acid; DMEM, Dulbecco's Modified Eagle's Medium; DMP, 2,3-dimercaptopropanol; DMSO, dimethylsulfoxide; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FCS, fetal calf serum; FGF, fibroblast growth factor; GSAO-F, 4-(*N*-(*S*-(*N*-(3-(fluorescein-5-carbamoylmethylthio)propanoyl)glutathionyl)acetyl)amino)phenylarsenoxide; FXAO, a mixture of 4-(*N*-(6-(fluorescein-5-carboxamido)hexanoyl)amino)phenylarsenoxide and 4-(*N*-(6-(fluorescein-6-carboxamido)hexanoyl)amino)phenylarsenoxide; GlcAO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-D-glucosamine; GluAO, *N*-(3-(4-arsenosophenylcarbamoylmethylthio)-propanoyl)-L-glutamic acid; GSAA, 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsonic acid; GSAO, 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide; GSAO-B, 4-(*N*-(*S*-(*N*-(6-(*N*-(biotinoyl)-amino)hexanoyl)amino)hexanoyl)glutathionyl)acetyl)amino)phenylarsenoxide; GSH, reduced glutathione; HDMVEC, human dermal microvascular endothelial cell; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid); HIV, human immunodeficiency virus; HRP, horse-radish peroxidase; HUVEC, human umbilical vein endothelial cell; Ig, immunoglobulin; MPB, 3-(*N*-maleimidylpropionyl)biocytin; PAO, phenylarsenoxide; pAPAO, 4-aminophenylarsenoxide; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PDI, protein disulfide isomerase; PVDF, polyvinyldiethylene fluoride; SCID, severe combined immunodeficient; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; SSB, sulfosuccinimidobiotin; TCR, T cell receptor; TNB, 5-thio-2-nitrobenzoate dianion; VEGF, vascular endothelial cell growth factor.

Brief Description of the Drawings

Figure 1. Structure of GSAO and GSAA.

Figure 2. Schematic representation of the irreversible inhibition of a redox active protein by initial binding of an arsenoxide group with a dithiol of the protein, followed by alkylation of the active site of the protein.

Figure 3. Synthesis of GSAO. Schematic representation of the synthesis of GSAO showing the stereochemistry and the numbering scheme used in the discussion of the 2D ^{1}H–^{13}C HMBC NMR spectrum.

Figure 4. Assignment of the Structure of GSAO. An expansion of the ^{1}H–^{13}C HMBC spectrum of GSAO in DCl/D_2O, showing the aliphatic region. The spectrum shows any long-range

heteronuclear ($^{1}H-^{13}C$) coupling as crosspeaks, in line with the corresponding ^{1}H and ^{13}C signals along the horizontal and vertical axes. The boxed crosspeaks correspond to $^{1}H-^{13}C$ coupling between the C7 and C11 methylenes, confirming that alkylation by BRAO has occurred on the glutathione sulfur atom. Peaks and crosspeaks marked "i" are due to impurities; one-bond crosspeaks corresponding to the C9 methylene and the C2 methine are also observable as doublets due to incomplete filtering by the HMBC pulse sequence.

Figure 5. Schematic representation of the synthesis of GSAA

Figure 6. Schematic representation of the synthesis of GSAO-B

Figure 7. Schematic representation of the synthesis of GSAO-F

Figure 8: Schematic representation of the synthesis of GSAO-Cy™5.5

Figure 9. Synthesis of FXAO

Figure 10. General synthetic route for compounds of the invention where A is a hydrophilic amine. Examples of R-NH_2 include D-glucosamine; L-aspartic acid; L-glutamic acid; and L-cysteic acid.

Figure 11. Titration of DMP or cysteine with GSAO. DMP (15 μM corresponding to 30 μM thiols) or cysteine (28 μM) was incubated with GSAO (0 - 50 μM) for 10 minutes. DTNB (950 μM) was then added and the reactions incubated for a further 10 minutes. The concentration of thiol in the reactions was determined from the absorbance of the TNB dianion at 412 nm. GSAO bound to DMP and prevented interaction of the dithiol with DTNB while any interaction of GSAO with cysteine was displaced by DTNB.

Figure 12. Interaction of GSAO with synthetic, peptide and protein dithiols. DMP (A) (15 μM), 6,8-thioctic acid (B) (11 M), dithiothreitol (C) (8.5 μM), TrpCysGlyProCysLys (D) (4.6 μM), TrpCysGlyHisCysLys (E) (5.5 μM) or thioredoxin (F) (4.1 μM corresponding to 20.6 μM thiols) was incubated with GSAO (0 - 56 μM) for 10 minutes. DTNB (950 μM) was then added and the reactions incubated for a further 10 minutes. The concentration of thiol in the reactions was determined from the absorbance of the TNB dianion at 412 nm. The solid lines represent the best non-linear least squares fit of the data to Equation 1 (A to E) or Equation 2 (F) and have been drawn using the parameter estimates in Table 1. The dotted line in part A represents a simulated titration assuming an infinite affinity of GSAO for the dithiol. GSAO bound to both synthetic, peptide and protein dithiols with dissociation constants in the range 130 nM to 1.4 μM.

Figure 13. Inhibition of thioredoxin by GSAO. Thioredoxin (1 μM) was incubated with GSAO (0, 1 or 10 μM) for 10 minutes at room temperature in 20 mM Hepes, 0.14 M NaCl, pH 7.4 buffer. The 70 kDa fibronectin fragment (10 μg per mL) was added and incubated for 5 minutes at room temperature. The reactions were labelled with MPB (100 μM) for 10 minutes at 37°C. The MPB was quenched with GSH (200 μM) for 10 minutes at 37°C followed by iodoacetamide (400 μM) for 10 minutes at room temperature. The MPB-labelled 70 kDa fragment was resolved on 5-15%

SDS-PAGE, transferred to PVDF membrane and the MPB detected by blotting with streptavidin peroxidase. The positions of Mr markers are shown at left.

Figure 14. Interaction of GSAO-B with PDI and thioredoxin. **A** Structure of GSAO-B. **B** Purified human recombinant PDI (5 μM), human recombinant thioredoxin (5 μM) or bovine serum albumin (5 μM) was incubated with dithiothreitol (10 μM) for 60 minutes at room temperature to ensure that the active site disulfide(s) of PDI and thioredoxin were in the reduced dithiol form. GSAO-B (100 μM) or GSAO-B and DMP (400 μM) was then added and the reactions incubated for 30 minutes at room temperature. The labelled PDI (lanes 1 and 2), thioredoxin (lanes 3 and 4) and albumin (lane 5) (75 pmoles) was resolved on 4-16% SDS-PAGE, transferred to PVDF membrane, and blotted with streptavidin-peroxidase to detect the GSAO-B label. The positions of M_r markers are shown at left.

Figure 15. Comparison of the cytotoxic effects of a membrane permeable versus substantially membrane-impermeable trivalent arsenicals. BAE cells (5 x 10^3 cells) were seeded in wells of 96 well plates and allowed to attach for 24 hours at 37°C and 5% CO_2. The cells, which were ~ 80% confluent, were washed two times with PBS and incubated with 100 μl of complete medium containing increasing concentrations of either PAO, GSAO, AspAO, GluAO, Cys*AO, GlcAO or FXAO (0 to 0.6 mM) for 24 hours at 37°C and 5% CO_2. The cells were then washed two times with PBS to remove non-adherent cells and adherent cells were counted using methylene blue as described by Oliver et al. (1989). The data points are the mean and SE of triplicate wells.

Figure 16. Identification of endothelial cell surface proteins that contain closely spaced dithiol(s). **A** The surface of HMEC-1 or HUVE cells (2 x 10^6 cells in 0.4 mL) was labelled with GSAO-B (100 μM) for 30 minutes at room temperature in the absence (lanes 1 and 3) or presence of DMP (400 μM) (lane 2). The endothelial cells were lysed and the lysate from both incubations was resolved on 4-15% SDS-PAGE, transferred to PVDF membrane, and blotted with streptavidin peroxidase to detect the GSAO-B label. The results represent labelling of 3 x 10^4 HMEC-1 (lanes 1 and 2) of HUVE cells (lane 3). The positions of M_r markers are shown at left. **B** Densitometry profile of the surface labelled proteins (lanes 1 and 3). The apparent M_r's of the individual proteins are indicated.

Figure 17. PDI was one of the GSAO-B labelled proteins on the endothelial cell surface. HUVEC (5 x 10^6 cells in 0.75 mL) were labelled with GSAO-B (100 μM) for 30 minutes at 37°C in the absence (lanes 1) or presence of DMP (400 μM) (lane 2). The cells were lysed and incubated with streptavidin-agarose beads to collect the biotin-labelled proteins. The labelled proteins were resolved on 4-15% SDS-PAGE, transferred to PVDF membrane, and blotted with anti-PDI monoclonal antibodies. The results represent labelling of 5 x 10^6 endothelial cells. The positions of M_r markers are shown at left.

Figure 18. Inhibition of proliferation and reduction in viability of BCE cells by GSAO. 15,000 (part A and B) or 50,000 (part C and D) BCE cells per well were seeded into gelatinised 24-well culture plates and allowed to attach for 24 hr (part A, B and D) or 72 hr (part C). The media was then replaced with DMEM containing 5% BCS and 0 to 1 mM GSAO or GSAA and either 1 ng per mL FGF-2 (part A) or 10 ng per mL VEGF (part B). In part D, the media was replaced with DMEM containing 5% BCS and 1ng per mL FGF-2 and either 10 μM GSAO or GSAA. In part C, the media was replaced with DMEM containing 5% BCS and 0 to 1 mM GSAO or GSAA. Cells were cultured for 72 hr in parts A, B and C or for discrete times up to 48 hr in part D and then dispersed and counted. The dotted line in parts A, B and C indicate the cell number in control wells containing DMEM and 5% BCS which represented no to limited proliferation. The dotted line in part D represents no change in cell number over the 48 hr. The data points and errors represent the mean and range of duplicate wells.

Figure 19. Effect of GSAO on proliferation of non-endothelial cells. 15,000 BxPC-3 (part A), HT1080 (part B), 3T3 (part C) or BVSM (part D) cells per well were seeded into gelatinised 24-well culture plates and allowed to attach for 24 hr. The media was then replaced with DMEM containing 0 to 1mM GSAO or GSAA and either 5% BCS (part D) or 5% FCS (parts A, B and C). Cells were cultured for 72 hr and then dispersed and counted. The dotted lines indicate the cell number in control wells containing DMEM and 2% BCS (part D) or 2% FCS (parts A, B and C) which represented no to limited proliferation. The data points and errors represent the mean and range of duplicate wells.

Figure 20. Effect of GSAO on proliferation of bone marrow progenitor cells. Human bone marrow cells were incubated in semi-solid agar containing 20% BCS, 1 ng per mL IL-3 and 0 to 100 μM GSAA or GSAO for 12 days. Granulocyte/macrophage colonies of 40 or more cells were counted under an inverted microscope. The data points and errors represent the mean and SE of quadruplicate cultures.

Figure 21. GSAO perturbs endothelial cell tube formation in Matrigel. Matrigel (100 μl) was added to wells of 96 well plates and allowed to gel for 60 minutes at 37°C. HDMVE cells (10,000 cells per well) in complete media containing 0.1, 1 or 100 μM GSAA or GSAO were seeded onto the Matrigel and the plates were incubated in 5% CO_2, 37°C. The phase contrast micrographs were taken after 18 hours incubation. GSAO perturbed tube formation by HDMVE cells in Matrigel (bottom panel). Effects were apparent at 0.1 μM concentration and marked at 100 μM. GSAA at the same concentrations had no apparent effect on tube formation (top panel).

Figure 22. Inhibition of CAM angiogenesis by GSAO. Fertilised 3 day-old white Leghorn eggs were cracked, the embryos placed in petri dishes and incubated for 3 days. Methylcellulose discs containing 5, 10 or 50 μg of either GSAA or GSAO were then applied to the CAM of individual

embryos and incubated for 48 hr. The CAMs were scored for no obvious effect or inhibition of angiogenesis as defined by avascular zones. Photographs of CAM's after incubation with discs containing 10 μg of either GSAA (top) or GSAO (bottom) is shown in the left hand panel. The dotted circle indicates the placement of the disc. The bar graph in the right hand panel shows the number out of 5 zones positive for angiogenesis inhibition at 5, 10 or 50 μg of GSAO per pellet. GSAA did not inhibit CAM angiogenesis up to 50 μg per pellet.

Figure 23. Inhibition of adjuvant-induced arthritis in rats by GSAO. Arthritis in the rat paws became apparent between days 11-13 post-induction and the disease peaked at around days 17-21. Administration of 2 mg/kg/rat/day GSAO effectively blocked the development of arthritis measured by either arthritis severity score (top left), gait (top right), paw volume (bottom left) or weight loss (bottom right). The control compound, 4-(*N*-(*S*-glutathionylacetyl)amino)-carboxylic acid (GSCA), had no effect on arthritis development. There were no signs or symptoms of toxicity of GSAO for the rats. The data points are the mean and standard error of 10 rats.

Figure 24. Effect of GSAO treatment on histological features of arthritis in the rat ankles. Administration of 2 mg/kg/rat/day GSAO significantly reduced the periarticular inflammation and pannus formation in the rat ankles. The bars and errors are the mean and SE of 20 rat ankles (10 rats per group).

Best Mode of Performing the Invention

1. Trivalent Organoarsenical Derivatives

For the treatment of arthritis, the present invention utilises compounds wherein at least one arsenoxide or arsenoxide equivalent moiety is linked, with or without the incorporation of a spacer group, to at least one substantially cell membrane impermeable pendant group, the pendant group being substantially cell membrane-impermeable by virtue of being charged at physiological pH or being hydrophilic in nature.

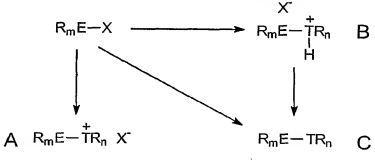
In a preferred form of the compounds utilised in the present invention, the compound is a dithiol reactive compound, such as a compound which contains a trivalent arsenical as outlined above. Redox active proteins are often characterised by one or more pairs of closely spaced dithiols which undergo cycles of oxidation and reduction. Trivalent arsenicals have high affinity for closely spaced dithiols, forming dithioarsine derivatives (Adams et al., 1990). Monothiols react very poorly with trivalent arsenicals because two monothiols are required to form the dithioarsine derivative. The process is entropically disfavoured and the binding of the second monothiol is usually sterically restricted.

As a specific example of a substantially cell membrane impermeable group which constitutes a suitable pendant group for the purposes of the present invention, glutathione is a tripeptide that is constitutively secreted by mammalian cells but is not substantially taken up by these cells.

A preferred compound for use in the method of the present invention capitalises on this substantially cell-membrane impermeability feature of glutathione to use glutathione as an essentially inert carrier of an arsenoxide group having the ability to bind to closely spaced dithiols of redox active proteins. In this manner, glutathione is used in the present invention to deliver the arsenoxide group to the mammalian cell surface, but to substantially inhibit passive entry of said moiety into cells.

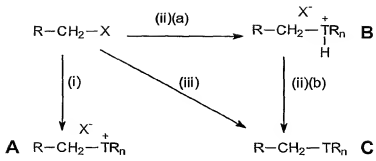
The compounds of general formulae (I)-(VI) as defined in the first embodiment of the invention and those in which the arsenoxide group (-As=O) is replaced by an arsenoxide equivalent, may be prepared by methods known generally in the art. Suitable methods for the synthesis of compounds of formulae (I-VI) and intermediates thereof are described, for example, in Houben-Weyl, *Methoden der Organischen Chemie;* J. March, *Advanced Organic Chemistry,* 4th Edition (John Wiley & Sons, New York, 1992); D. C. Liotta and M. Volmer, eds, *Organic Syntheses Reaction Guide* (John Wiley & Sons, Inc., New York, 1991); R. C. Larock, *Comprehensive Organic Transformations* (VCH, New York, 1989), H. O. House, *Modern Synthetic Reactions* 2nd Edition (W. A. Benjamin, Inc., Menlo Park, 1972); N. S. Simpkins, ed., *100 Modern Reagents* (The Royal Society of Chemistry, London, 1989); A. H. Hains *Methods for the Oxidation of Organic Compounds* (Academic Press, London, 1988) and B. J. Wakefield *Organolithium Methods* (Academic Press, London, 1988).

Example reaction schemes to illustrate the generic formation of linkers of compounds useful in the method of the present invention are shown in the following schemes.



wherein E represents an electrophilic site; and m, n are integers greater than or equal to 0.

The scheme below shows a starting molecule RCH_2X, where R represents the rest of the molecule to which the $-CH_2X$ group is attached. X represents a leaving group, for example, a halogen or RSO_3–, which is displaced by the nucleophile TR_n. Nucleophiles attack at electrophilic sites, resulting in the formation of a new covalent bond between the nucleophilic and electrophilic species. In the scheme below, the methylene carbon atom is the electrophilic site, and the overall reaction can be described as one of nucleophilic substitution.

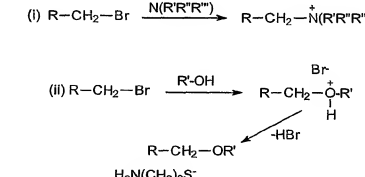


There are three simple variations on the above scheme, as illustrated by reactions (i) to (iii): in this reaction, the attacking nucleophile is represented by the uncharged molecule TR_n, which displaces the leaving group X, giving the product **A** which has a positive charge formally localised on T.

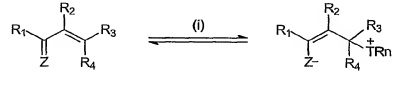
the first step (a) of this reaction involves the attacking nucleophile HTR_n displacing the leaving group X, giving the ionic product **B** initially, followed by loss of H^+ in step (b) to give the uncharged product **C**.

in this reaction, product **C** is formed directly by use of TR_n^- as the nucleophile.

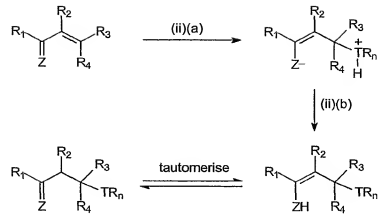
In all three reactions (i) to (iii), X is lost as X^-, and atom T must have a lone pair of electrons. Shown below are general examples of each of the reactions (i) to (iii). Note that reaction (iii) is analogous to the formation of GSAO from BRAO and GSH.



Alternatively, the reaction may be between a nucleophile and, for example, an α,β-unsaturated ketone (when Z=O) (or aldehyde when Z=O and R_1 = H) as illustrated in the following schemes. For example, where the nucleophile is TR_n:



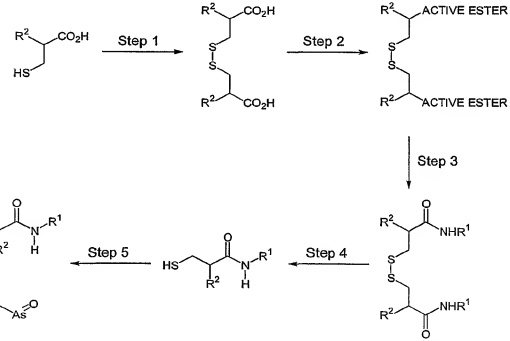
where the nucleophile is HTR_n



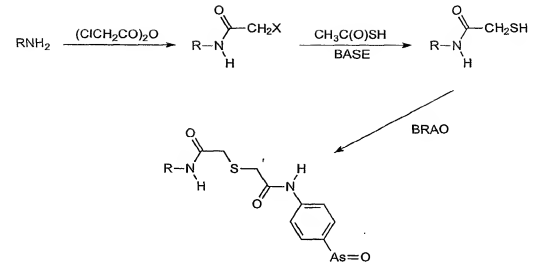
where the nucleophile is TR_n–.

wherein Z is selected from the group consisting of O, S, NR, or +N(R)(R').

A typical example of a general synthetic route for preparing hydrophilic amine compounds of the invention is represented in the following scheme, wherein the reagent in step 5 has been exemplified as BRAO:



Still more typically, hydrophilic amine compounds useful in the methods of the present invention can be prepared according to the general scheme outlined below which has been exemplified using BRAO in the final step and wherein X is a halogen or other suitable leaving group.



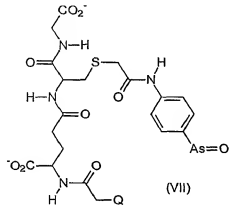
In respect of the above schemes, one skilled in the art would recognise that the various reagents and reactants can be routinely modified in order to synthesise a range of compounds defined by Formula (I) for use in the method of the present invention. The compounds described herein can be lyophilised for storage and reconstituted prior to use.

In a typical synthesis of a preferred compound for use in the method of the present invention, glutathione may be reacted with BRAO under conditions favourable to the formation of a covalent bond between the free thiol of glutathione and the chemical entity to which the arsenoxide is attached. Reactions involving nucleophilic attack by the glutathione thiol will, in general, require alkaline conditions. Electrophilic attack of some reactive species on the glutathione sulfur atom may be carried out; in general this would likely require acidic conditions. The structure of GSAO and the corresponding arsonic acid compound GSAA are shown in Fig. 1.

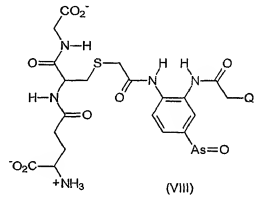
Typically, the compounds for use in the method of the present invention are inhibitors of redox active proteins by virtue of an ability to bind dithiols. Redox active proteins may contain two closely spaced thiols which can reversibly form a disulfide bond. A proposed mode by which the compounds of the invention inhibit these proteins is by the arsenoxide or arsenoxide equivalent binding to the reduced (dithiol) form of the protein. Such binding may be essentially irreversible or essentially reversible under physiological conditions. If the binding is essentially irreversible under physiological conditions, the protein is permanently inhibited from redox-cycling between the dithiol and disulfide states (ie. it is irreversibly inactivated, or inhibited).

Alternatively, if the binding of the arsenoxide or arsenoxide equivalent to the protein dithiol is essentially reversible under physiological conditions, inhibition will not be permanent. Accordingly, compounds for use in the method of the present invention may include having a substituent which can act as an alkylating agent, attached to the $(XBX')_nB'$ linker or the substantially cell membrane impermeable group, A. The alkylating group may be brought into the vicinity of one of the active site dithiols of the protein by the reaction of the arsenical group with the dithiol of the protein. The

alkylating group may then react with the dithioarsine-protein intermediate, thereby permanently inhibiting the protein and preventing redox-cycling. An example of this mode of irreversible inhibition resulting from alkylation is represented in Fig. 2. Compounds having an alkylating agent attached to the $(XBX')_nB'$ linker or the substantially cell-membrane impermeable group, A, are exemplified by the structural formulae (VII) and (VIII) below, wherein the pendant group A is glutathione:



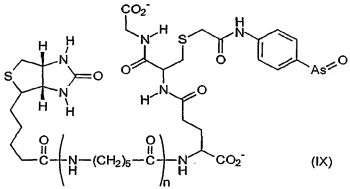
(VII)



(VIII)

wherein Q is a leaving group.

Other compounds suitable for use in the invention are modified through the glutamyl α-amino nitrogen of glutathione, for example, with a detectable group, such as biotin, a fluorophore, or a group comprising a transition element. An example of such a compound is GSAO-B, a biotin-linked derivative of GSAO, according to the following formula (IX):

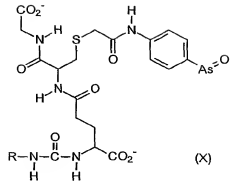


(IX)

wherein n = 1 or 2.

A method of synthesis of GSAO-B is provided in Example 1(c) and illustrated in Fig. 6.

An alternative preferred compound for use in the method of the present invention is one in which a desired modifying group may be attached through the glutamyl α-amino nitrogen of glutathione as represented in the following formula (X):



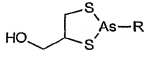
(X)

wherein R is any desired modifying group.

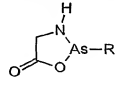
Typically, R may be selected from the group consisting of hydrogen, halogen, hydroxy, amino, nitro, carboxy, alkoxy, alkyl, and aryl.

1.1 Stability of the trivalent arsenicals towards oxidation

Arsenoxides (R-As=O) have been shown not to possess an arsenic-oxygen double bond as is usually written, but are likely to exist either as cyclic polymers (containing As-O-As linkages) or, more likely, as the hydrate R-As(OH)$_2$, an organoarsonous acid, in aqueous solution (Doak and Freedman, 1970; Knoch et al, 1995). Solutions of organoarsenicals such as GSAO and BRAO are deactivated over time by oxidation. This oxidation can be slowed in three ways; removal of dissolved O_2 from solutions containing the arsenoxides, lowering the pH of these solutions, or by addition of glycine to the solutions. Glycine is routinely used to prevent oxidation of the stock solutions of the trivalent organoarsenicals. Similar to the reaction between 2,3-dimercaptopropanol and R-As(OH)$_2$ in which a 5-membered dithioarsonite (XI) is formed, reaction of glycine with R-As(OH)$_2$ is thought to give a 5-membered cyclic 1,3,2-oxazarsolidin-5-one (XII).



(XI)



(XII)

2. Treatment and/or Prevention of Arthritis

The present invention provides a method for the treatment and/or prevention of arthritic diseases and disorders of vertebrates.

Typically, the vertebrate is selected from the group consisting of human, non-human primate, murine, bovine, ovine, equine, caprine, leporine, avian, feline and canine. More typically, the vertebrate is human, non-human primate or murine. Even more typically, the vertebrate is human.

The compounds described herein, or pharmaceutical formulations thereof, may be used in the prevention and/or treatment of arthritis, examples of which include the following: calcific periarthritis, enteropathic arthritis, chronic arthritis, gout, hand osteoarthritis, hip arthritis, knee osteoarthritis, thumb arthritis, Jaccoud's arthritis, juvenile osteoarthritis, oligoarthritis, polyarthritis, peripheral arthritis, psoriatic arthritis, rheumatoid arthritis and septic arthritis.

A number of proteins are known to stimulate endothelial cell growth and movement, including epidermal growth factor, angiogenin, estrogen, the fibroblasts growth factors (FGFs), and vascular endothelial growth factor (VEGF). Anti-angiogenic factors include interferon, thrombospondin, platelet factor 4, tissue inhibitors of metalloproteinase-1 and -2, interleukin 12, angiostatin and endostatin.

There are ten distinct proteins on the endothelial cell surface with molecular masses of between 12 and 138 kDa that bind GSAO, and this is as exemplified in Example 3(a) and Figure 16. This finding suggests that the endothelial cell surface supports redox events in certain proteins. Perturbation of these events has consequences for endothelial cell biology, such as effects on proliferation of endothelial cells, as outlined in general in Example 3. More specifically, examples 3(c) and 3(d) and Figures 18-21 indicate that GSAO was a selective inhibitor of proliferation and tube formation of endothelial cells in culture. GSAO also inhibited new blood vessel formation in the chick chorioallantoic membrane, (Example 3(o) and Figure 22).

2.4 Pharmaceutical and/or Therapeutic Formulations

Typically, for medical use, salts of the compounds described herein, which are suitable for use in the present invention will be pharmaceutically acceptable salts; although other salts may be used in the preparation of the compound or of the pharmaceutically acceptable salt thereof. By pharmaceutically acceptable salt it is meant those salts which, within the scope of sound medical judgement, are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

For instance, suitable pharmaceutically acceptable salts of the compounds for use in the present invention may be prepared by mixing a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, methanesulfonic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, phosphoric acid, acetic acid, oxalic acid, carbonic acid, tartaric acid, or citric acid with compounds described herein. Suitable pharmaceutically acceptable salts of compounds suitable for use in the present invention therefore include acid addition salts.

For example, S. M. Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, *66*:1-19. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base

function with a suitable organic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, asparate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

Also suitable for use in the present invention are prodrugs of the compounds described herein. Typically, prodrugs will be functional derivatives of the compounds described herein which are readily converted *in vivo* to the therapeutically active compounds as described herein which are useful in the present invention. Typical procedures for the selection and preparation of prodrugs are known to those of skill in the art and are described, for instance, in H. Bundgaard (Ed), *Design of Prodrugs*, Elsevier, 1985.

Single or multiple administrations of pharmaceutical compositions comprising the compounds suitable for use in the present invention can be carried out with dose levels and pattern being selected by the treating physician. Regardless, a pharmaceutical composition used according to the present invention should provide a quantity of the pharmaceutically active compound as hereinbefore described, sufficient to effectively treat the patient.

One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of the compounds hereinbefore described which would be required to treat or prevent the disorders and diseases of the present invention. Generally, an effective dosage is expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg

body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

Alternatively, an effective dosage may be up to about 500mg/m^2. Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m^2, preferably about 25 to about 350mg/m^2, more preferably about 25 to about 300mg/m^2, still more preferably about 25 to about 250mg/m^2, even more preferably about 50 to about 250mg/m^2, and still even more preferably about 75 to about 150mg/m^2.

In relation to GSAO, an effective dosage is expected to be in the range of about 0.0001 mg to about 100 mg GSAO per kg body weight per 24 hours, preferably about 0.001 mg to about 100 mg GSAO per kg body weight per 24 hours, more preferably about 0.01 mg to about 50 mg GSAO per kg body weight per 24 hours, even more preferably about 0.1 mg to about 20 mg GSAO per kg body weight per 24 hours, even more preferably still about 0.1 mg to about 10 mg GSAO per kg body weight per 24 hours. Typically the treatment would be for the duration of the condition.

Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages of compounds used according to the present invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the nature of the particular vertebrate being treated. Also, such optimum conditions can be determined by conventional techniques.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the compound described herein given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

When used for the treatment of arthritis according to the present invention, compounds corresponding to Formula (I) as hereinbefore described may be administered alone. However, it is generally preferable that the compound be administered as a pharmaceutical formulation which may include a pharmaceutically acceptable carrier, diluent and/or adjuvant.

The carriers, diluents and adjuvants must be "acceptable" in terms of being compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Examples of pharmaceutically and veterinarily acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose

or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrridone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

A preferred form of pharmaceutical composition suitable for use in the present invention comprises an effective amount of a compound as hereinbefore described, such as GSAO, together with a pharmaceutically acceptable carrier, diluent and/or adjuvant as shown in Example 5.

The pharmaceutical compositions may be administered by standard routes. In general, the compositions may be administered by the intra-articular, topical, transdermal, intraperitoneal, oral, rectal, or parenteral (e.g., intravenous, intraspinal, subcutaneous or intramuscular) route. Typically, the administration route is intra-articular. Still generally, the compositions for use in the invention may be in the form of a capsule suitable for oral ingestion, in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation.

The pharmaceutical compositions may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. Formulations in liposome form may contain, in addition to a compound as hereinbefore described, stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*, the contents of which is incorporated herein by reference.

For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2-propylene glycol.

Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colourings agents. When used in

capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration of the capsule.

Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulfite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides, or mixtures thereof.

Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or –laurate, and the like.

The emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above, or natural gums such as guar gum, gum acacia or gum tragacanth.

The topical formulations suitable for use in the methods of the present invention, comprise an active ingredient together with one or more acceptable carriers, and optionally any other therapeutic ingredients.

Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose.

Drops suitable for use in the methods of the present invention may comprise sterile aqueous or oily solutions or suspensions. These may be prepared by dissolving the active ingredient in an aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container and sterilised. Sterilisation may be achieved by: autoclaving or maintaining at 90°C-100°C for half an hour, or by filtration, followed by transfer to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions suitable for use in the methods of the present invention include those suitable for application to the skin. A lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those described above in relation to the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturiser such as glycerol, or oil such as castor oil or arachis oil.

Creams, ointments or pastes suitable for use in the methods of the present invention are semi-solid formulations of the active ingredient for external application. They may be prepared by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols.

The formulation may incorporate any suitable surface active agent such as an anionic, cationic or non-ionic surface active such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

Compositions for parenteral administration in accordance with the present invention will commonly comprise a solution of a compound described herein or a cocktail thereof dissolved in

an acceptable carrier, such as water, buffered water, 0.4% saline, and 0.3% glycine etc, wherein such solutions are sterile and relatively free of particulate matter.

Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

Pharmaceutical compositions suitable for use in accordance with the methods of the present invention may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. Formulations in liposome form may contain, in addition to a compound as hereinbefore described, stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*, the contents of which is incorporated herein by reference.

Depending on the intended result, pharmaceutical compositions can be administered for prophylactic and/or therapeutic treatments. In a therapeutic application, compositions are administered to a patient already suffering from an arthritic disease, in an amount sufficient to cure or at least partially arrest the disease and its complications. In prophylactic applications, compositions containing a compound as hereinbefore described or a cocktail thereof, are administered to a patient not already in a diseased state to enhance the patient's resistance.

Typically, the compounds suitable for use in the invention may be used in combination with other known treatments, such as anti-inflammatory therapeutic agents. For example, glucocorticosteroids, such as prednisolone and methylprednisolone, are often-used anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs (NSAIDs) are also used to suppress inflammation. NSAIDs inhibit the cyclooxygenase (COX) enzymes, COX-1 and COX-2, which are central to the production of prostaglandins produced in excess at sites of inflammation. In addition, the inflammation-promoting cytokine, tumor necrosis factor α (TNFα), is associated with multiple inflammatory events, including arthritis, and first generation anti-TNFα therapies are being used clinically.

The invention will now be described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Examples

Example 1

Synthesis of Compounds Corresponding to General Formula (I)

The following chemicals were purchased and used without further purification: phenylarsenoxide, bromoacetyl bromide, sulfur dioxide, d_6-dimethylsulfoxide, deuterium oxide, L-aspartic acid, L-glutamic acid, D-glucosamine hydrochloride (Aldrich, Castle Hill, NSW); methanol, 98% sulfuric acid, 48% hydrobromic acid, 37% hydrochloric acid (Ajax, Auburn, NSW); dichloromethane, potassium hydroxide, sodium hydrogen carbonate, sodium hydroxide (BDH, Kilsyth, VIC); P-2 Gel extra fine 1,800 MW cut-off (Bio-Rad, Hercules, CA); 2,3-dimercaptopropanol (DMP), L-cysteic acid (Fluka, Castle Hill, NSW); thionyl chloride (Merck, Darmstadt, Germany); 6,8-thioctic acid, dithiothreitol, dimethylsulfoxide, 5,5'-dithiobis(2-nitrobenzoic acid), ethylenediaminetetraacetic acid, *N*-(2-hydroxyethyl)piperazine-*N*'-(2-ethanesulfonic acid), glutathione, sodium carbonate, sodium chloride, sodium iodide (Sigma, Castle Hill, NSW); *p*-arsanilic acid (Tokyo Kasei Kogyo, Tokyo, Japan); glycine (ICN, Aurora, Ohio). 3-(fluorescein-5-carbamoylmethylthio)propanoic acid, succinimidyl ester (fluorescein-5-EX, SE), 6-(fluorescein-*x*-carboxamido)hexanoic acid (mixed isomers: x = 5 or 6), succinimidyl ester (5(6)-SFX), and 6-((6-((biotinoyl)amino)hexanoyl)amino)hexanoic acid, succinimidyl ester (biotin-XX, SE) were obtained from Molecular Probes, Eugene, Oregon. Cy™5.5 monofunctional dye was obtained from Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK. All other reagents were of analytical grade.

Instrumentation - 1D and 2D NMR spectra were obtained using a Bruker DPX300 nuclear magnetic resonance spectrometer, with ^1H and ^{13}C detected at 300.17 MHz and 75.48 MHz, respectively. UV-visible absorbances were recorded on a Molecular Devices Thermomax Plus (Palo Alto, CA) microplate reader.

Preparation of acidified deuterium oxide - Fresh thionyl chloride was cautiously added to an excess of deuterium oxide. After evolution of SO_2 had ceased, the resulting solution (0.6 ml) was added to GSAO (ca 50 mg) in a 5 mm NMR tube. This sample was used to obtain the NMR spectra.

Example 1(a)

Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide (GSAO)

The total synthesis of GSAO is represented schematically in Fig. 3.

Synthesis of 4-(*N*-(bromoacetyl)amino)phenylarsonic acid (BRAA)

Sodium carbonate (40.14 g, 378.7 mmol) was added to water (200 mL) and stirred at room temperature until all solids had dissolved. To the stirred carbonate solution was added *p*-arsanilic acid (29.99 g, 138.2 mmol), portionwise, and the volume of the solution made up to 300 mL with addition of more water. The solution (pH 10 to 11) was allowed to stir for 30 mins, and if necessary, was filtered to remove any undissolved solid before being refridgerated for 2 to 3 hours. The solution was transferred to a separating funnel and ice chips were added. Bromoacetyl

bromide (15 mL, 34.76 g, 172.1 mmol) was diluted in dichloromethane (50 mL) and approximately half of the dichloromethane solution was added carefully to the cold aqueous solution. The mixture was cautiously shaken, with frequent venting to avoid excessive build up of pressure. After 1 to 2 mins, the evolution of carbon dioxide had subsided, and more vigorous shaking was undertaken. The remaining portion of bromoacetyl bromide was carefully added and the procedure repeated. When the reaction was over, the solution was found to be pH 7. The lower dichloromethane layer was discarded, and the aqueous layer transferred to a 1 L flask and carefully acidified by dropwise addition of 98% sulfuric acid. Complete precipitation of the white product required addition of acid until the solution was approximately pH 1. The crude product was collected and dried at the pump, typically in yields of 50% to 75%. ^{1}H-NMR (d_6-DMSO): δ 4.09 (s, 2H), 7.73 (d, J = 9 Hz, 2H), 7.83 (d, J = 9 Hz, 2H), 10.87 (s, 1H). ^{13}C-NMR (d_6-DMSO): δ 30.53, 119.97, 127.34, 131.56, 143.08, 166.00 ppm.

Synthesis of 4-(*N*-(bromoacetyl)amino)phenylarsenoxide hydrate (BRAO.xH_2O)

Into a 3-necked 500 mL round-bottomed flask was placed BRAA (12.15 g, 36 mmol). The solid was dissolved with swirling in a mixture of methanol (75 mL) and hydrobromic acid (48%, 75 mL), giving a transparent yellow solution. The solution was filtered to remove residual solids. Sodium iodide (0.20 g, 1.3 mmol) was added as a catalyst, whereupon the colour of the solution darkened to orange-brown, then sulfur dioxide gas was slowly (ca. 2 bubbles per second) passed through the stirred solution for approximately 2.5 hours. The resultant white precipitate was collected using a Büchner funnel, giving the product (17.43 g) as a damp white solid. The activity of a solution made by dissolving a portion of the solid (40.7 mg) in deoxygenated DMSO (800 μL) was determined to be 56 mM (see below). Hence, the molecular weight of BRAO.xH_2O is 908.5, that is, 35% w/w BRAO and 65% w/w H_2O. Therefore, the "anhydrous" weight of the BRAO product was 35% of 17.43 g, that is, 6.10 g (19 mmol, 53% yield). ^{1}H-NMR (d_6-DMSO): δ 4.85 (s, 2H), 7.78 (d, J = 9 Hz, 2H), 7.86 (d, J = 9 Hz, 2H), 11.36 (s, 1H). ^{13}C-NMR (d_6-DMSO): δ 30.55, 119.22, 130.52, 140.04, 145.04, 165.52 ppm.

Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide (GSAO)

DMSO (10 mL) was deoxygenated by passing a stream of nitrogen gas through it for a few minutes, and used to dissolve BRAO.xH_2O (1.00 g, 2.48 mmol active arsenoxide). Glutathione (1.15 g, 3.74 mmol, 1.5 eq) was dissolved in 0.5 M bicarbonate buffer, pH 9.6 (35 mL), and added to the solution of BRAO.xH_2O in DMSO. The total volume was made up to 50 mL with 0.5 M bicarbonate buffer, and the solution gently agitated at room temperature overnight. Cautious neutralisation with 37% hydrochloric acid, followed by lyophilisation gave a white powdery product, which could be dissolved in water leaving no residual solid. The active arsenoxide concentration of

the resultant solution was found to be 49.6 mM, determined using the DMP/DTNB assay (see below).

The product was purified using gel-filtration (P-2 Gel extra fine, 1.8 kDa cutoff, 50 g) on a 130 mL column, using 20 mM Hepes, 0.14 M NaCl, 1 mM EDTA, pH 7.4 buffer as the eluant at a flow rate of 0.10 mL/min. A total of 144 mL was collected (72 fractions of 2 mL) and monitored by UV (λ 214nm). Four peaks, A, B, C and D, were resolved. Peaks B and C showed activity in the DTNB/DMP assay (see below), and were assigned as GSAO and unreacted BRAO, respectively. Peaks A and D were tentatively assigned as the oxidation products GSAA and BRAA (the oxidation product of BRAO), respectively (see below). Unreacted GSH was also detected (using DTNB) in the fractions corresponding to Peak A. The fractions corresponding to peak B were combined and deoxygenated with nitrogen gas to give a solution of GSAO (15 mM, approximately 12 mL). ^{1}H-NMR (D_2O): δ 1.93 (q, J = 7 Hz, 2H), 2.35 (t, J = 8 Hz, 2H), 2.84 (dd, J = 14 Hz, J = 9 Hz, 1H), 3.05 (dd, J = 14 Hz, J = 5 Hz, 1H), 3.35 (s, 2H), 3.58 (t, J = 6 Hz, 1H), 3.64 (d, J = 2 Hz, 2H), 4.48 (dd, J = 9 Hz, J = 5 Hz, 1H), 7.44 (d, J = 8 Hz, 2H), 7.58 (d, J = 8 Hz, 2H). ^{13}C-NMR (D_2O): δ 25.93, 31.16, 33.53, 36.01, 42.97, 52.83, 53.89, 121.29, 129.97, 138.77, 144.09, 170.90, 171.73, 173.75, 174.68, 175.76 ppm.

2D NMR spectroscopy was also used to confirm the structure of GSAO. A series of ^{1}H and ^{13}C NMR spectra, ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC, were all found to be consistent with the structure proposed in Fig. 3. Considered together, all of the spectra permitted the unambiguous assignment of all carbon and non-exchangeable hydrogen atoms. An expansion of the ^{1}H–^{13}C HMBC spectrum of GSAO, showing the aliphatic region, is shown in Fig. 4. The ^{1}H–^{13}C HMBC technique correlates coupled ^{1}H and ^{13}C nuclei, but filters out directly bonded nuclei. This means that ^{1}H and ^{13}C nuclei that are separated by two, three, or (sometimes) four bonds appeared as crosspeaks in the spectrum. Fig. 4 shows that C11 is only strongly coupled to H7 (referring to the protons attached to C7), while C7 is strongly coupled to H11 in addition to H6. This confirms that the GSH sulfur was successfully alkylated with BRAO.

Example 1(b)

Synthesis of 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsonic acid (GSAA)

The synthesis of GSAA is represented schematically in Fig. 5.

BRAA (1.00 g, 2.96 mmol) and glutathione (1.36 g, 4.44 mmol, 1.5 eq) were dissolved in 0.5 M bicarbonate buffer, pH 9.6 (50 mL), and the solution gently agitated at room temperature overnight. Lyophilisation gave a white powdery product which was freely soluble in water, leaving no solid residue. The product was purified by gel-filtration on a 570 mL column (2.5 x 117 cm) of Bio-Gel P-2 extra fine (BioRad, Hercules, CA) using deionised water as the eluant at a flow rate of

0.1 mL per min. The product (GSAA) eluted from the column in a position corresponding to Peak A in the purification of GSAO.

Example 1(c)

Synthesis of 4-(*N*-(*S*-(*N*-(6-((6-((biotinoyl)amino)hexanoyl)amino)hexanoyl)glutathionyl)acetyl)-amino)phenylarsenoxide (GSAO-B)

The synthesis of GSAO-B is represented schematically in Fig. 6.

GSAO (0.13 g) was dissolved in 0.5 M sodium bicarbonate buffer (5 mL, pH 8.5) and the concentration of active arsenical in the resultant solution was determined to be 39 mM. The buffered arsenical solution (4.2 mL, containing 165 μmol active arsenical) was added to a solution of biotin-XX, SE (100 mg, 176 μmol) in DMSO (1 mL), the mixture inverted a few times and then incubated at 4°C for 4 hours. Glycine (17.5 mg, 233 μmol) was added and the mixture kept at 4°C overnight. The concentration of trivalent arsenical in the GSAO-B product was determined to be 31 mM and the solution was used without further modification.

Example 1(d)

Synthesis of 4-(*N*-(*S*-(*N*-(3-(fluorescein-5-carbamoylmethylthio)propanoyl)glutathionyl)acetyl)-amino)phenylarsenoxide (GSAO-F)

The synthesis of GSAO-F is represented schematically in Fig. 7.

A solution of fluorescein-5-EX succinimidyl ester (2.4 mg, 4.1 μmol) in DMSO (240 μL) was added to GSAO (33.8 mM) in Mes buffer, pH 5.5 (5 mM, 473 μL), and the mixture was diluted with bicarbonate buffer, pH 9 (0.5 M, 3.287 mL) and allowed to stand at room temperature for 80 mins. The yellow solution was then diluted with glycine (100 mM) in PBS (4 mL), and allowed to stand at room temperature overnight. The final solution contained trivalent arsenical (2.00 mM) and glycine (50 mM).

Example 1(e)

Synthesis of GSAO-Cy™5.5

The synthesis of GSAO-Cy™5.5 is represented schematically in Fig. 8.

A solution of Cy™5.5 (266 nmol) in bicarbonate buffer, pH 9 (0.5 M, 968 μL) was mixed with a solution of GSAO (33.8 mM) in Mes buffer, pH 5.5 (5 mM, 32 μL), and allowed to stand at room temperature for 80 mins. The blue solution was then diluted with glycine (100 mM) in PBS (1 mL), and allowed to stand at room temperature overnight. The final solution contained trivalent arsenical (0.54 mM) and glycine (50 mM).

Example 1(f)

Synthesis of 4-aminophenylarsenoxide (pAPAO)

The synthesis of pAPAO is represented schematically in Fig. 9.

Hydrobromic acid (48%, 35 mL) was added to a suspension of p-arsanilic acid (7.76 g, 35.8 mmol) in methanol (35 mL) with stirring. Sodium iodide (ca 0.05 g, 0.33 mmol) was added then the solution was placed in a water bath (room temperature). Sulfur dioxide was bubbled through the stirred solution at a rate of about 2 bubbles per second. After 2 hours, a white precipitate had formed. The reaction was allowed to proceed overnight, by which time most of the solid had redissolved. The remaining solid was collected and dried at the pump, yielding 3.89 g. A solution of pAPAO (24 mg) in DMSO (1 mL) was prepared, and the active concentration of trivalent arsenical was determined (see determination of GSAO activity). From this, the product was found to consist of 3.57 mmol active arsenical (10% yield).

Example 1(g)

Synthesis of a mixture of 4-(*N*-(6-(fluorescein-5-carboxamido)hexanoyl)amino)-phenylarsenoxide and 4-(*N*-(6-(fluorescein-6-carboxamido)hexanoyl)amino)-phenylarsenoxide (FXAO)

The synthesis of FXAO is represented schematically in Fig. 9.

A solution of a mixture of 6-(fluorescein-5-carboxamido)hexanoic acid, succinimidyl ester and 6-(fluorescein-6-carboxamido)hexanoic acid, succinimidyl ester (4.9 mg, 8.29 µmol) in DMSO (107 µL) was added to pAPAO (22 mM) in DMSO (193 µL). The mixture was diluted with 0.5 M bicarbonate buffer, pH 9 (700 µL) then allowed to stand at room temperature for 80 mins. The yellow solution was diluted with glycine (100 mM) in PBS (1 mL), and allowed to stand at room temperature overnight. The final solution contained trivalent arsenical (2.12 mM) and glycine (50 mM).

Example 1(h)

Synthesis of 3,3'-dithiobis(propanoic acid)

A scheme outlining the synthesis is provided in Fig. 10.

Sodium carbonate (64.0 g, 604 mmol) was added to water (300 mL) and the solution stirred at room temperature until all solids had dissolved. 3-Mercaptopropanoic acid (50 mL, 60.9 g, 574 mmol) was added dropwise to the stirred carbonate solution at such a rate that evolution of carbon dioxide did not become excessively vigorous. Dichloromethane (50 mL) was added to the resulting suspension, followed by portionwise addition of iodine (71.44 g, 281 mmol, 0.49 eq). During the addition of iodine, vigorous evolution of carbon dioxide was observed. The mixture was transferred to a 500 mL separating funnel, and the lower organic layer discarded. The aqueous layer was filtered, and cautiously acidified to pH 1 with 98% sulfuric acid. The precipitate was collected and

dried at the pump to give the product (43.4 g, 72% yield). ^{1}H-NMR (d_6-DMSO): δ 2.59 (t, J = 7 Hz, 2H), 2.86 (t, J = 7 Hz, 2H), 12.25 (s, 1H). ^{13}C-NMR (d_6-DMSO): δ 33.4, 34.0, 173.0 ppm.

Example 1(i)

Synthesis of 3,3'-dithiobis(propanoic acid, succinimidyl ester)

A scheme outlining the synthesis is provided in Fig. 10.

3,3'-Dithiobis(propanoic acid) (9.5 g, 45.2 mmol) was dissolved in a mixture of acetone (600 mL) and dichloromethane (600 mL) with stirring at room temperature. *N*-Hydroxysuccinimide (12.68 g, 110.2 mmol, 2.44 eq) was dissolved in the solution, then 1,3-dicyclohexylcarbodiimide (25.9 g, 125.5 mmol, 2.78 eq) was cautiously added. The mixture was allowed to stir at room temperature for 24 hours, after which time the solution was vacuum filtered and the residual solid discarded. The solvent was removed from the filtrate under vacuum, and the oily residue was re-dissolved in dichloromethane (ca 200 mL). The solution was reduced in volume (ca. 50 mL) and cooled, giving the product as a colourless, crystalline solid (8.90 g, 49% yield). ^{1}H-NMR (d_6-DMSO): δ 2.80 (s, 8H), 3.05 (m, 8H). ^{13}C-NMR (d_6-DMSO): δ 25.8, 30.7, 32.3, 167.9, 170.4 ppm.

Example 1(j)

Synthesis of the disulfide of disodium *N*-(3-mercaptopropanoyl)-L-aspartate

A scheme outlining the synthesis is provided in Fig. 10.

L-Aspartic acid (0.42 g, 3.16 mmol) was mixed with sodium hydrogen carbonate (0.50 g, 5.95 mmol, 1.9 eq), then water (1 mL) was added. The sodium hydrogen carbonate was added to quench the acidic hydrogens present in L-aspartic acid. When evolution of carbon dioxide had subsided, the mixture was diluted with 0.5 M bicarbonate buffer, pH 9 (19 mL, 9.5 mmol). All solids dissolved with swirling and the solution was found to be pH 9. 3,3'-Dithiobis(propanoic acid, succinimidyl ester) (0.247 M in DMSO, 5 mL, 1.24 mmol) was added dropwise to the aqueous carbonate solution with stirring. Immediately, a white precipitate appeared, but upon vigorous shaking it redissolved. After 24 hours, during which time the mixture was periodically shaken, dichloromethane (1 mL) was added, and the mixture was again allowed to stand for 24 hours with periodic shaking. The solution, (pH 9) was acidified to pH 7 by the dropwise addition of 32% hydrochloric acid. The product was precipitated by the slow, dropwise addition of the solution to a stirred beaker of absolute ethanol (300 mL) at room temperature, giving a fluffy white precipitate which was collected by filtration and dried under vacuum, giving the product as a white solid (0.37 g, 53% yield). ^{13}C-NMR (D_2O): δ 33.2, 34.8, 37.2, 50.9, 175.6, 176.0, 176.4 ppm.

Example 1(k)

Synthesis of the disulfide of disodium *N*-(3-mercaptopropanoyl)-L-glutamate

A scheme outlining the synthesis is provided in Fig. 10.

The procedure used was the same as for the disulfide of disodium *N*-(3-mercapto-propanoyl)-L-aspartate, using L-glutamic acid (0.44 g, 2.99 mmol) and sodium hydrogen carbonate (0.55 g, 6.55 mmol, 2.2 eq). The product was obtained as a white solid (0.61 g, 87% yield). ^{13}C-NMR (D_2O): δ 28.5, 33.2, 34.1, 35.0, 55.2, 173.6, 179.2, 181.2 ppm.

Example 1(l)

Synthesis of the disulfide of disodium *N*-(3-mercaptopropanoyl)-L-cysteate

A scheme outlining the synthesis is provided in Fig. 10.

The procedure used was the same as for the disulfide of disodium *N*-(3-mercaptopropanoyl)-L-aspartate, using L-cysteic acid (0.59 g, 3.15 mmol) and sodium hydrogen carbonate (0. 56 g, 6.67 mmol, 2.1 eq). The product was obtained as a white solid (0.68 g, 79% yield). ^{13}C-NMR (D_2O): δ 33.5, 35.7, 50.8, 52.3, 174.1, 178.9 ppm.

Example 1(m)

Synthesis of the disulfide of disodium *N*-(3-mercaptopropanoyl)-D-glucosamine

A scheme outlining the synthesis is provided in Fig. 10.

The procedure followed that for the amino acid derivatives described above, using D-glucosamine hydrochloride (0.67 g, 3.11 mmol), except that no additional sodium hydrogen carbonate was added initially, and the product was precipitated with AR acetone (300 mL) instead of ethanol. The product was obtained as a white solid (0.67 g, 81% yield). ^{13}C-NMR (D_2O): δ 33.8, 35.4, 35.7, 39.3, 54.6, 57.2, 61.1, 61.2, 70.4, 70.6, 71.1, 72.0, 74.3, 76.4, 91.3, 95.4 ppm.

Example 1(n)

Synthesis of *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-aspartic acid (AspAO)

A solution of 70.0 mM BRAO in DMSO (1.68 mL, 118 μmol) was mixed with a solution of the disulfide of disodium *N*-(3-mercaptopropanoyl)-L-aspartate (0.37 g) dissolved in 0.5 M bicarbonate buffer, pH 9 (6.72 mL, 3.4 mmol). Added to the solution was 0.69 M triphenylphosphine in DMSO (1.1 mL, 760 μmol). There was an immediate precipitation of what was presumed to be insoluble triphenylphosphine, so DMSO (1 mL) was added, and the mixture shaken thoroughly, and left to stand at room temperature overnight. The mixture was filtered, and acidified to pH 4 with the dropwise addition of 32% hydrochloric acid, giving 7.5 mL of solution. The active concentration of trivalent arsenic was found to be 17.5 mM (using the same method used to determine the active concentrations of BRAO and GSAO).

Example 1(o)

Synthesis of *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-glutamic acid (GluAO)

The procedure used was the same as for *N*-(3-(4-arsenosophenylcarbamoylmethylthio)-propanoyl)-L-aspartic acid, using 2.63 mL (184 μmol) of 70.0 mM BRAO in DMSO, the disulfide of disodium *N*-(3-mercaptopropanoyl)-L-glutamate (0.61 g), 0.5 M bicarbonate buffer, pH 9 (10.52 mL, 5.3 mmol), and 0.69 M triphenylphosphine in DMSO (1.7 mL, 1.2 mmol). In this case, DMSO (2 mL) was added to the precipitated mixture before leaving overnight. The active concentration of trivalent arsenic was found to be 15.4 mM (10.3 mL of solution).

Example 1(p)

Synthesis of *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-L-cysteic acid (Cys*AO)

The procedure used was the same as for *N*-(3-(4-arsenosophenylcarbamoyl-methylthio)propanoyl)-L-glutamic acid, using 2.70 mL (189 μmol) of 70.0 mM BRAO in DMSO, the disulfide of disodium *N*-(3-mercaptopropanoyl)-L-cysteate (0.68 g), 0.5 M bicarbonate buffer, pH 9 (10.80 mL, 5.4 mmol), and 0.69 M triphenylphosphine in DMSO (1.8 mL, 1.2 mmol). The active concentration of trivalent arsenic was found to be 17.5 mM (12.3 mL of solution).

Example 1(q)

Synthesis of *N*-(3-(4-arsenosophenylcarbamoylmethylthio)propanoyl)-D-glucosamine (GlcAO)

The procedure used was the same as for *N*-(3-(4-arsenosophenylcarbamoyl-methylthio)propanoyl)-L-glutamic acid, using 3.00 mL (210 μmol) of 70.0 mM BRAO in DMSO, the disulfide of disodium *N*-(3-mercaptopropanoyl)-D-glucosamine (0.67 g), 0.5 M bicarbonate buffer, pH 9 (11.96 mL, 6.0 mmol), and 0.69 M triphenylphosphine in DMSO (1.9 mL, 1.3 mmol). The active concentration of trivalent arsenic was found to be 13.5 mM (11.0 mL of solution).

Example 2

Assay and Reactivity of GSAO

Example 2(a)

Assay of BRAO, GSAO and GSAO-B

A stock solution of DMP (5 μL, 50 μmol) was dissolved in DMSO (995 μL), giving a concentration of 50 mM DMP. A second dilution of the 50 mM DMP stock solution (10 μL) in pH 7.0 buffer (0.1 M HEPES, 0.3 M NaCl, 1 mM EDTA) (990 μL) gave a working solution of 500 μM DMP. The activity of the arsenical could then be determined by the titration of varying amounts of arsenical against the DMP working solution (10 μL) in a 96-well microtitre plate, with the total volume made up to 195 μL by addition of buffer. After a 10 minute incubation at room temperature, during which time the solutions were agitated on a plate shaker, 5 μL of a 37.9 mM stock solution of DTNB (15 mg) in DMSO (1 mL) was added, and the plate incubated with shaking

for another 10 minutes. The absorbance at 412 nm due to the formation of the TNB dianion was measured using a Molecular Devices Thermomax Plus (Palo Alto, CA) microplate reader. The extinction coefficient for the TNB dianion at pH 7.0 is 14,150 $M^{-1}cm^{-1}$ at 412 nm (Riddles et al., 1983).

GSAO bound to DMP and prevented interaction of the dithiol with DTNB while any interaction of GSAO with cysteine was displaced by DTNB (Fig. 11). This result confirmed the dithiol selectivity of GSAO.

Example 2(b)

Interaction of GSAO with synthetic, peptide and protein dithiols

Recombinant human thioredoxin produced in *E. coli* was from American Diagnostica, Greenwich, CT. The hexapeptides, TrpCysGlyProCysLys and TrpCysGlyHisCysLys, were from Auspep, Parkville, Australia.

Binding of GSAO to dithiols was measured from loss of thiols using the DTNB assay described above. The dissociation constant, K_d, for GSAO binding to dithiols was determined by incubating increasing concentrations of GSAO, I, with a fixed dithiol concentration, $[S]_T$, and measuring the remaining dithiol using DTNB. Note that the concentration of dithiol equals half the TNB concentration. The concentration of dithiol.GSAO complex, SI, as a function of the total GSAO concentration, $[I]_T$, is described by Equation 1 (Hogg and Jackson, 1990),

$$[SI] = 0.5.\{([S]_T + x.[I]_T + K_d) - (([S]_T + x.[I]_T + K_d)^2 - 4.[S]_T.x.[I]_T)^{0.5}\} \quad (1)$$

where x is a factor that, when multiplied together with $[I]_T$, will yield the active concentration of GSAO. Data was fit to equation 1 by non-linear least squares regression with K_d and x the unknown parameters (Scientist software, Micromath, Salt Lake City, Utah). x was 1 ± 0.2 for all the dithiols tested.

Thioredoxin contains 5 accessible thiols that react with DTNB (Holmgren, 1989). Titration of thioredoxin with GSAO resulted in a decrease from 5 to 3 thiols upon complex formation. The dissociation constant, K_d, for GSAO binding to thioredoxin was determined by incubating increasing concentrations of GSAO, I, with a fixed thioredoxin thiol concentration, $[S]_T$, and measuring the remaining thiol groups using DTNB. Note that the concentration of thiol groups equals the TNB concentration. The concentration of thioredoxin thiol.GSAO complex, SI, as a function of the total GSAO concentration, $[I]_T$, is described by Equation 2,

$$[S]_T = 2.[SI] + 2.[S]_D + [S]_M \quad (2a)$$

$$[SI] = 0.5.\{([S]_D + x.[I]_D + K_d) - (([S]_D + x.[I]_T + K_d)^2 - 4.[S]_D.x.[I]_T)^{0.5}\} \quad (2b)$$

where $[S]_D$ is the concentration of thioredoxin dithiol that complexes with GSAO and $[S]_M$ is the concentration of the remaining thiol groups. Data was fit to equation 2 by non-linear least

squares regression with K_d and x the unknown parameters (Scientist software, Micromath, Salt Lake City, Utah). x was 1.5 ± 0.2 for thioredoxin.

The small synthetic dithiols, DMP, 6,8-thioctic acid and dithiothreitol, formed high affinity complexes with GSAO (Fig. 12A, B and C, Table 1). The affinity for GSAO decreased as the size of the ring structure with the arsenic of GSAO increased. For instance, the two thiols of DMP are on adjacent carbon atoms which form a five-membered ring with the GSAO arsenic. The affinity of GSAO for the dithiols decreased from a dissociation constant of 130 nM for a five membered ring with DMP to 420 nM for a seven-membered ring with dithiothreitol.

GSAO also bound with high affinity to both peptide and protein dithiols. The two peptides, TrpCysGlyProCysLys (Holmgren, 1989) and TrpCysGlyHisCysLys (Gilbert, 1997), correspond to the active site sequences of thioredoxin and PDI, respectively. Both peptides bound GSAO with dissociation constants of approximately 1 μM (Fig. 12D and E, Table 1). There were 15 atoms in the ring structure of the peptides with the arsenic of GSAO. Despite this large ring structure the dissociation constant for binding of GSAO was only double that for binding of GSAO to dithiothreitol. This result implied that the secondary structure of the peptides brought the two Cys thiols into close proximity which enabled them to complex with the trivalent arsenical. GSAO bound to the active site dithiol of thioredoxin with a dissociation constant of 370 ± 180 nM (Fig. 12F, Table 1), which was ~4-fold higher affinity than that of GSAO binding to the thioredoxin active site hexapeptide, 1,420 ± 450 nM. This result implied that the distance between the active site thiols in thioredoxin was closer than their distance in the hexapeptide.

Considered together, these results indicated that GSAO selectively binds proteins containing closely spaced thiols. To identify these proteins on the cell surface a biotin moiety was attached through a spacer arm to the primary amino group of the γ-glutamyl residue of GSAO. Incorporation of GSAO-B into proteins could be assessed by measuring the biotin using streptavidin-peroxidase.

Table 1. Dissociation constants for binding of GSAO to synthetic and protein dithiols.

Dithiol	Ring Size[a]	Dissociation Constant, nM
2,3-Dimercapto-1-propanol	5	130 ± 40[b]
6,8-Thioctic acid	6	200 ± 50
Dithiothreitol	7	420 ± 80
TrpCysGlyProCysLys	15	1,420 ± 450
TrpCysGlyHisCysLys	15	870 ± 270
Thioredoxin	15	370 ± 180

a number of atoms in the ring structure with the arsenic of GSAO.

b errors are 1 SD.

Example 2(c)

Inhibition of thioredoxin activity by GSAO

It was observed that thioredoxin reduced one or more protein disulfide bonds in the 70 kDa N-terminal fragment of fibronectin. Incubation of 1 μM GSAO with 1 μM thioredoxin for 10 minutes in Hepes buffered saline resulted in ~50% inhibition of thioredoxin-mediated reduction of the fibronectin fragment, whereas incubation with 10 μM GSAO completely inhibited thioredoxin activity (Figure 13).

Example 2(d)

Interaction of GSAO-B with PDI and thioredoxin

Human recombinant PDI and thioredoxin bound GSAO-B (Fig. 14). Recombinant human protein disulfide isomerase (PDI) was produced in *E. coli* and purified according to Jiang et al. (1999). In the experiment, purified PDI, thioredoxin or albumin as negative control were incubated with a 2-fold molar excess of dithiothreitol for 60 minutes to ensure that the active site disulfides of PDI and thioredoxin were in the reduced dithiol state. The proteins were then incubated with GSAO-B or GSAO-B and a 4-fold molar excess of DMP for 30 minutes. Equivalent moles of the labelled proteins were resolved on SDS-PAGE, transferred to PVDF membrane, and blotted with streptavidin-peroxidase to detect the GSAO-B label. Samples were resolved on 4-15% SDS-PAGE under non-reducing conditions (Laemmli, 1970) and transferred to PVDF membrane. Proteins were detected by Western blot using an anti-PDI murine monoclonal antibody (Jiang et al., 1999) (used at 2 μg per mL). Rabbit anti-mouse horseradish peroxidase conjugated antibodies (Dako Corporation, Carpinteria, CA) were used at 1:2000 dilution. GSAO-B-labelled proteins were blotted with streptavidin peroxidase (Amersham, Sydney, NSW) used at 1:1000 dilution. Proteins were visualised using chemiluminescence (DuPont NEN, Boston, MA) according to the manufacturer's instructions. Chemiluminescence films were analysed using a GS-700 Imaging Densitometer and Multi-Analyst software (Bio-Rad, Hercules, CA).

Both PDI and thioredoxin incorporated GSAO-B but albumin did not. The higher M_r band in lane 1 of Fig. 14B was a small amount of aggregated PDI in the preparation (Jiang et al., 1999). It is noteworthy that the density of labelling of PDI was approximately twice that of thioredoxin which is consistent with the two active site dithiols of PDI versus the one of thioredoxin.

Example 2(e)

Comparison of the effects of hydrophobic, hydrophilic or charged trivalent arsenicals on endothelial cell viability

Bovine aortic endothelial (BAE) cells (Hotchkiss et al., 1998) were seeded in wells of 96 well plates overnight, washed and then incubated with complete medium containing increasing concentrations of either the membrane permeable PAO, or the substantially membrane-

impermeable GSAO, AspAO, GluAO, Cys*AO, GlcAO or FXAO. After 24 hours incubation the adherent cells were counted.

PAO was very toxic for BAE cells with an IC_{50} for viability of < 0.1 μM (Fig. 15). The viability IC_{50}'s for GSAO, AspAO, GluAO, Cys*AO, GlcAO and FXAO were ~100 μM, < 1 μM, < 10 μM, < 10 μM, < 1 μM and > 200 μM, respectively (Fig. 15). GSAA had no significant effect on BAE viability up to 10 mM concentration (not shown). This result demonstrated that limiting the entry of the trivalent arsenical into the cell by attaching it to charged (GSH, Asp, Glu, cysteic acid) or hydrophilic (glucosamine, fluorescein-X) pendants reduced toxicity by 10- to >2,000-fold.

Example 2(f)

GSAO is substantially membrane-impermeable

GSAO does not cross the plasma membrane to any significant extent. Human T cells (A3.01) or adherent BAE cells were incubated with complete medium containing 50 μM GSAO and ~100,000 counts per minute of tritiated GSAO for 1 or 72 hours. Tritiated GSAO was prepared exactly as described for GSAO except that glycine-2-^{3}H-glutathione (NEN, Boston, MA) was used in place of cold glutathione. The cells were washed and lysed by two cycles of freezing and thawing. The cytosolic constituents were collected by differential centrifugation (Ausbel et al., 2000) and the tritiated GSAO was measured. Only 0.5% of the total tritiated GSAO was found in the T cell cytosol and 0.04% in the BAE cytosol after 1 hour. Moreover, only 4.5% of the GSAO penetrated the T cell membrane after 72 hours of culture.

Example 3

Effect of GSAO on Angiogenesis

Example 3(a)

Identification of endothelial cell surface proteins that contain closely spaced dithiol(s)

Human umbilical vein endothelial cells (HUVEC) (Wall et al., 1978) and the human dermal microvascular endothelial cell line, HMEC-1 (Ades et al., 1992), were harvested and cultured as indicated. Endothelial cells (5 x 10^6) were detached from culture flasks using 5 mM EDTA in PBS at 37°C, washed 3 times with PBS, resuspended in PBS containing 100 μM of GSAO-B in the absence or presence of 400 μM DMP, and incubated for 30 minutes at room temperature. The cells were washed 3 times with 1 mL of PBS, resuspended in 0.2 mL of ice cold 50 mM Tris/HCl, pH 8 buffer containing 0.5 M NaCl, 1% Triton X-100, 10 μM leupeptin, 2 mM PMSF (Sigma Chemical Company, St. Louis, MO), 5 mM EDTA and 10 μM aprotinin (Bayer Australia Ltd., Sydney, NSW), and sonicated on ice. On some occasions the cell lysates were incubated with streptavidin-agarose (Sigma, Castle Hill, NSW) beads (25 μL of packed beads in a total volume of 1 mL) for 60 minutes at 4°C with rotary mixing. Bound proteins were washed 5 times with 50 mM

Tris/HCl, pH 8 buffer containing 0.15 M NaCl and 0.05 % Triton X-100, resolved on SDS-PAGE, transferred to PVDF membrane, and the GSAO-B-labelled proteins detected by Western blot.

There were approximately ten proteins on the endothelial cell surface that incorporated GSAO-B (Fig. 16A). The molecular masses of these proteins varied from 12 to 138 kDa (Fig. 16B). The intensity of labelling of the proteins varied considerably which probably reflected their abundance on the cell surface, although it may also have reflected differences in the affinity of GSAO for the protein dithiols. The labelling was specific as there was effectively no incorporation of GSAO-B in the presence of DMP.

Example 3(b)

PDI was one of the GSAO-B labelled proteins on the endothelial cell surface

BAE cells were labelled with GSAO-B in the absence or presence of DMP, lysed and incubated with streptavidin-agarose beads to collect the biotin-labelled proteins. The labelled proteins were eluted from the beads, resolved on SDS-PAGE, transferred to PVDF membrane, and blotted with anti-PDI polyclonal antibodies.

The results shown in Fig. 17 indicate that PDI was one of the proteins on the endothelial cell surface that incorporated GSAO-B. There was no labelling of PDI in the presence of DMP which is supported by the results of Fig. 14. The higher M_r band in lane 1 of Fig. 17 was some aggregated PDI (see Fig. 14B).

Example 3(c)

Inhibition of proliferation of endothelial cells by GSAO

BCE (Folkman et al. 1979), BAE, BxPC-3 (ATCC, Bethesda, MD), HT1080 (Jiang et al., 1999), 3T3 (ATCC, Bethesda, MD) and BVSM (Hogg et al., 1997) cells were harvested and cultured as indicated. Cells (0.5 mL of 30,000 or 100,000 cells per mL) were seeded into gelatinized 24-well culture plates (Corning Costar, Corning, NY) in Dulbecco's Modified Eagle's Medium (DMEM, JHR Bioscience, Lenexa, KS) containing 10% fetal calf serum (FCS, Intergen Comp., Purchase, NY) and 1% Glutamine Pen-Strep (GPS, Irvine Scientific, Santa Ana, VA) and allowed to attach for 24 hr in 10% CO_2 at 37°C. The media was then replaced with DMEM and 1% GPS containing 0 to 1 mM GSAO or GSAA and either 5% bovine calf serum (BCS, HyClone, Logan, UT) and 1 ng per mL FGF-2 (Genzyme, Cambridge, MA) or 5% BCS and 10 ng per mL VEGF (Genzyme, Cambridge, MA) for BCE cells, 5% or 10% BCS for BAE cells, 5% BCS for BVSM cells, and 5% FCS for BxPC-3, HT1080 and 3T3 cells. Cells were cultured for 0 to 72 hr in 10% CO_2 at 37°C then dispersed in trypsin/EDTA (GibcoBRL, Grand Island, NY), resuspended in Coulter balanced electrolyte solution and counted with a Z1 Coulter counter (Coulter Corp., Miami, FL). In all experiments there were two control wells containing DMEM with 1% GPS and either 5%

BCS for BCE cells, 2% BCS for BAE and BVSM cells, or 2% FCS for BxPC-3, HT1080 and 3T3 cells. These wells represented no to limited proliferation.

GSAO inhibited the proliferation and reduced the viability of BCE cells in culture (Fig. 18). In contrast, GSAA had only a marginal effect on proliferation at the highest concentration used, 1 mM, and no significant effect on viability. Inhibition of proliferation by GSAO was independent of whether the cells were stimulated with either fibroblast growth factor-2 (FGF-2) (Fig. 18A) or vascular endothelial cell growth factor (VEGF) (Fig. 18B). The IC_{50} for inhibition of proliferation in response to FGF-2 or VEGF was ~0.1 μM and ~0.05 μM, respectively (Table 2). GSAO also reduced the viability of BCE cells with IC_{50}'s of ~10 μM and ~3 μM in the presence of FGF-2 or VEGF, respectively (Table 2). The anti-proliferative versus viability effects of GSAO were separated by measuring viability of confluent cultures of cells. For example, Fig. 18C shows the effects of GSAO on the viability of a near confluent culture of BCE cells. In this experiment there was < 10 % increase in cell number after 72 hr of culture. The time dependence of the effect of GSAO on proliferation and viability of BCE cells is shown in Fig. 18D. A concentration of 10 μM GSAO was chosen as this was the IC_{50} for decrease in viability (Table 2). There was no decrease in viability after 8 hours. The cell number declined thereafter.

GSAO also inhibited proliferation and reduced viability of BAE cells (not shown). GSAA did not effect either proliferation or viability. The IC_{50} values for inhibition of proliferation and reduction in viability by GSAO were in the same range as for BCE cells (Table 2). It is noteworthy that the IC_{50} for inhibition of proliferation of BAE cells was ~0.02 μM when 5% FCS was the mitogen versus ~0.2 μM when 10% FCS was used.

GSAO did not affect the proliferation of the human cancer cell lines, BxPC-3 or HT1080, nor murine 3T3 fibroblasts or bovine vascular smooth muscle (BVSM) cells (Fig. 19). GSAO did affect the viability of all these cells with an IC_{50} of ~40 μM (Table 2). This value was 4-13 times higher than the IC_{50}'s for reduction in viability of endothelial cells. GSAA had no significant effect on proliferation or viability of these cells up to a concentration of 1 mM.

Table 2. Effects of GSAO on proliferation and viability of cultured cells

Cell	Mitogen	Proliferation IC_{50}, μM[a]	Viability IC_{50}, μM[b]
BCE	5% BCS + 1 ng/mL FGF-2	~0.1	~10
BCE	5% BCS + 10 ng/mL VEGF	~0.05	~3
BAE	5% BCS	~0.02	~8
BAE	10% BCS	~0.2	~10
BxPc3	5% FCS	No effect	~40
HT1080	5% FCS	No effect	~40

BVSM	5% BCS	No effect	~40
3T3	5% FCS	No effect	~40

a The IC_{50} for inhibition of proliferation of BCE and BAE cells was calculated as the concentration of GSAO that reduced the extent of proliferation by half from the cell number in the absence of GSAO to the cell number in wells containing 5% BCS for BAE cells and 2% BCS for BAE cells in a 72 hr incubation (dotted lines in Fig. 18).

b The IC_{50} for reduction in viability of BCE and BAE cells was calculated as the concentration of GSAO that reduced the cell number by half in assays where cells were >90% confluent at the beginning of the 72 hr incubation (for example, see Fig. 18C). The IC_{50} for reduction in viability of all other cells was calculated as the concentration of GSAO that reduced the cell number by half in a 72 hr incubation.

GSAO reduced the number of granulocyte/macrophage colonies in a 12 day human bone marrow culture assay with an IC_{50} of ~30 μM (Fig. 20). GSAA had no effect on colony numbers up to a concentration of 100 μM. Human bone marrow cells (~5 x 10^5 cells per mL) were added to semi-solid agar in DMEM containing 20% BCS, 5 ng per mL IL-3 and 0 to 100 μM GSAA or GSAO. The cultures were incubated for 12 days at 37°C and 5% CO_2 and clusters of 40 or more cells were counted under an inverted microscope according to Metcalf (1977).

These findings indicated that GSAO was a selective inhibitor of proliferating microvascular and macrovascular endothelial cells. The inhibitory effects were independent of whether the mitogen was either FGF-2 or VEGF. GSAO also reduced the viability of endothelial cells in culture in a time dependent manner. The half-maximal effects on viability were at least 4-times smaller than the effects on viability of other primary or transformed cells.

Example 3(d)

Inhibition of endothelial cell tube formation by GSAO

Endothelial cells arrange into tube-like structures when seeded onto three-dimensional matrices such as the extracellular matrix preparation, Matrigel, or collagen. The tubes can be considered to represent immature blood vessels. Matrigel (100 μl, Becton Dickinson, Bedford, MA) was added to wells of 96 well plates (Gibco BRL, Gaithersburg, MD) and allowed to gel for 60 minutes at 37°C. Human dermal microvascular endothelial (HDMVE) cells were harvested and cultured as described by Stathakis et al. (1997). HDMVE cells (8,000 cells per well) in 150 μl of M199 (Gibco BRL, Gaithersburg, MD) media containing 30% pooled human serum, 50 μg per mL Heparin (Sigma, St. Louis, MO), 100 μg per mL Endothelial Cell Growth Supplement (Gibco BRL, Gaithersburg, MD) and 0.1, 1 or 100 μM GSAA or GSAO were seeded onto the Matrigel and the plates were incubated for 18 hours in 5% CO_2, 37°C. Phase contrast micrographs of the wells were collected.

GSAO perturbed tube formation by HDMVE cells in Matrigel (Fig. 21). Effects were apparent at 0.1 μM concentration and marked at 100 μM. GSAA at the same concentrations had no apparent effect on tube formation.

Example 3(e)

Inhibition of chick chorioallantoic membrane (CAM) angiogenesis by GSAO

The chick CAM assay has been used for the detection and analysis of angiogenesis inhibition (Nguyen et al., 1994). Fertilised 3 day-old white Leghorn eggs (Spafas, Norwich, CT) were cracked, the embryos with intact yolks placed in 20 x 100 mm petri dishes and incubated for 3 days at 37°C and 3% CO_2 (Folkman, 1995). Methylcellulose (Fisher Scientific, Fair Lawn, NJ) discs containing 5, 10 or 50 μg of either GSAA or GSAO were then applied to the CAM of individual embryos and incubated for 48 hr at 37°C and 3% CO_2. The discs were made by desiccation of GSAA or GSAO in 10 μl of 0.45% methycellulose on teflon rods. The CAMs were observed using a stereomicroscope and scored for no obvious effect or inhibition of CAM angiogenesis as defined by avascular zones. On some occasions CAM blood vessels were injected with India ink and photographed.

GSAO inhibited angiogenesis in the CAM in a concentration-dependent manner (Fig. 22). Angiogenesis inhibition was defined as avascular zones 48 hr after implantation of methylcellulose pellets containing GSAO on the 6-day CAM (see left hand panel of Fig. 22). GSAA up to 50 μg per pellet had no effects on CAM angiogenesis. Neither GSAA nor GSAO had any apparent adverse effect on the wellbeing of the chick embryo.

Example 4

Treatment of Arthritis with GSAO

Example 4(a)

Inhibition of adjuvant-induced arthritis in rats by GSAO

Methods

Male 4-6 weeks old Dark Agouti rats weighing 150-200 g were obtained from Gilles Plains, South Australia. Arthritis was induced by injection of Freund's adjuvant (0.1 mL of 10 mg per mL heat killed and dried *Mycobacterium butyricum* suspension in paraffin oil and mannide monoleate; Difco Laboratories, Detroit, MI) intradermally into the tail base of anaesthetized rats. Rats were treated with 2 mg/kg/rat/day GSCA or GSAO in phosphate-buffered saline containing 100 mM glycine via 0.2 mL subcutaneous injection. The glycine minimizes oxidation of the GSAO. GSCA is the control compound for GSAO. Disease progress was monitored every 3-4 days post-adjuvant by measuring paw volume by plesthysmometry (Ufo Basile, Comerio, Italy), arthritis severity score (as judged by swelling, necrosis and redness), gait score and body weight (Binder and Walker, 1988).

Results

Arthritis in the rat paws became apparent between days 11-13 post-induction and the disease peaked at around days 17-21. This is consistent with other reports of this arthritis model (Binder and Walker, 1998). Administration of 2 mg/kg/rat/day GSAO effectively blocked the development of arthritis measured by either arthritis severity score, gait, paw volume or weight loss (Fig. 23). The control compound, GSCA, had no effect on arthritis development. There was no signs or symptoms of toxicity of GSAO for the rats. For instance, there was no difference in weight gain between GSCA or GSAO treated rats in the first 10 days post-adjuvant.

Example 4(b)

Effects of GSAO treatment on histological features of arthritis in rat ankles

Methods

The GSCA- and GSAO-treated rats described in Example 4(a) were sacrificed on day 18 by injection with Lethobarb. The ankles were removed, fixed in 10% formalin for 10 days, decalcified in 30% formic acid for an additional 5 days and examined histologically to assess joint damage. Longitudinal sections were prepared such that the dorsoventral faces of the tarsal, metatarsal and phalangeal joints, bones and soft tissue were presented. The specimens were embedded in wax and 4 μm sections were cut, mounted on glass slides and stained with haematoxylin and eosin. Sections were scored for periarticular inflammation (density of inflammatory cells), pannus formation (degree of pannus intrusion into the joint space) and periosteal reaction (extent of new bone formation). A subjective rating score from 1-8 was assigned for each parameter. A normal joint would score 0 and the maximum score in an arthritic joint would be 8.

Results

Administration of 2 mg/kg/rat/day GSAO significantly reduced the periarticular inflammation and pannus formation in the rate ankles (Fig. 24). Only a few of the GSCA-treated rats and none of the GSAO-treated rats exhibited signs of new bone formation. Pannus formation was advanced in the GSCA-treated rats, although there was only a limited number of inflammatory cells. This is not an uncommon finding in this model. There was little to no pannus formation in the GSAO-treated rats and almost no inflammatory cells.

Example 5 - Pharmaceutical Formulations

The compounds suitable for use in the present invention may be administered alone, although it is preferable that they be administered as a pharmaceutical formulation. The active ingredient may comprise, for topical administration, from 0.001% to 10% by weight, and more typically from 1% to 5% by weight of the formulation, although it may comprise as much as 10% by weight.

In accordance with the best mode of performing the invention provided herein, specific preferred pharmaceutical compositions for use in the present invention are outlined below. The following are to be construed as merely illustrative examples of formulations and not as a limitation of the scope of the present invention in any way.

Example 5(a) - Topical Cream Composition

A typical composition for delivery as a topical cream is outlined below:

GSAO 1.0 g
Polawax GP 200 25.0 g
Lanolin Anhydrous 3.0 g
White Beeswax 4.5 g
Methyl hydroxybenzoate 0.1 g
Deionised & sterilised Water to 100.0 g

The polawax, beeswax and lanolin are heated together at 60°C, a solution of methyl hydroxybenzoate is added and homogenisation achieved using high speed stirring. The temperature is then allowed to fall to 50°C. GSAO is then added and dispersed throughout, and the composition is allowed to cool with slow speed stirring.

Example 5(b) - Topical Lotion Composition

A typical composition for delivery as a topical lotion is outlined below:

GSAO 1.2 g
Sorbitan Monolaurate 0.8 g
Polysorbate 20 0.7 g
Cetostearyl Alcohol 1.5 g
Glycerin 7.0 g
Methyl Hydroxybenzoate 0.4 g
Sterilised Water about to 100.00 ml

The methyl hydroxybenzoate and glycerin are dissolved in 70 ml of the water at 75°C. The sorbitan monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at 75°C and added to the aqueous solution. The resulting emulsion is homogenised, allowed to cool with continuous stirring and the GSAO is added as a suspension in the remaining water. The whole suspension is stirred until homogenised.

Example 5(c) - Composition for Inhalation Administration

For an aerosol container with a capacity of 20-30 ml: a mixture of 10 mg of GSAO with 0.5-0.8% by weight of a lubricating agent, such as polysorbate 85 or oleic acid, is dispersed in a propellant, such as freon, and put into an appropriate aerosol container for either intranasal or oral inhalation administration.

Example 5(d) - Composition for Intra-articular Administration

A pharmaceutical composition of GSAO for intra-articular injection could be prepared to contain 1 mL sterile buffered water, and 1 mg of GSAO.

Example 5(e) - Capsule Composition

A pharmaceutical composition of GSAO in the form of a capsule may be prepared by filling a standard two-piece hard gelatin capsule with 50 mg of GSAO, in powdered form, 100 mg of lactose, 35 mg of talc and 10 mg of magnesium stearate.

Example 5(f) - Injectable Parenteral Composition

A pharmaceutical composition of GSAO in a form suitable for administration by injection may be prepared by mixing 1% by weight of GSAO in 10% by volume propylene glycol and water. The solution is sterilised by filtration.

Example 5(g) - Ointment Composition

A typical composition for delivery as an ointment includes 1.0g of GSAO, together with white soft paraffin to 100.0 g, dispersed to produce a smooth, homogeneous product.

References

Adams E, Jeter D, Cordes AW, Kolis JW. 1990. Chemistry of organometalloid complexes with potential antidotes: structure of an organoarsenic(III) dithiolate ring. Inorg Chem 29:1500-1503.

Ades EW, Candal FJ, Swerlick RA, George VG, Summers S, Bosse DC, Lawley TJ. 1992. HMEC-1: establishment of an immortalised human microvascular endothelial cell line. J Invest Dermatol 99:683-690.

Ausbel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, D. "Current Protocols in Molecular Biology, Supplement 29 – Overview of Genetic Reporter Systems" John Wiley and Sons Inc., New York, 2000

Binder, W. and Walker, J. S. Effect of the peripherally selective κ-opioid agonist, asimadoline, on adjuvant arthritis. *Brit. J. Pharmacol.* 124, 647-654 (1998).

Doak GO, Freedman LD. 1970. In: Seyferth D, ed. Organometallic compounds of Arsenic, Antimony, and Bismuth. New York, New York: Wiley-Interscience.

Folkman J. 1995. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nature Med 1:27-30.

Folkman J, Haundenschild CC, Zetter BR. 1979. Long-term culture of capillary endothelial cells. Proc Natl Acad Sci USA 76:5217-5221.

Gilbert HF. 1997. Protein disulfide isomerase and assisted protein folding. J Biol Chem 272:29399-29402.

Hayes, A.J. Angiogenesis in rheumatoid arthritis. *Lancet* **354**, 423-424, 1999

Hogg PJ, Stathakis P, Jiménez BM, Chesterman CN. 1997. Interaction of platelet-derived growth factor with thrombospondin 1: Dependence on the disulfide-bond arrangement in thrombospondin 1. Biochem J 326:709-716.

Holmgren A. 1989. Thioredoxin and glutaredoxin systems. J Biol Chem 264:13963-13966.

Hotchkiss KA, Matthias LJ, Hogg PJ. 1998. Exposure of the cryptic Arg-Gly-Asp sequence in thrombospondin 1 by protein disulfide isomerase. Biochim Biophys Acta 1388:478-488.

Huppa JB, Ploegh HL. 1998. The eS-Sence of -SH in the ER. Cell 92:145-148.

Jiang X-M, Fitzgerald M, Grant CM, Hogg PJ. 1999. Redox control of exofacial protein thiols/disulfides by protein disulfide isomerase. J Biol Chem 274:2416-2423.

Knoch F, Schmachtel T, Ullrich V. 1995. Crystal structure of 4-amino-1-arsenoso-benzene dihydrate, $H_2NC_6H_4As(OH)_2(H_2O)$. Z Krist 210:642.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Metcalf D. 1977. Hemopoietic colonies: in vitro cloning of normal and leukemic cells. Recent Results Cancer Res 61:12-31.

Nguyen M, Shing Y, Folkman J. 1994. Quantitation of angiogenesis and antiangiogenesis in the chick embryo chorioallantoic membrane. Microvascular Res 47:31-40.

Oliver MH, Harrison NK, Bishop JE, Cole PJ, Laurent GJ. 1989. A rapid and convenient assay for counting cells cultured in microwell plates: application for assessment of growth factors. J Cell Sci 92:513-518.

Riddles PW, Blakeley RL, Zerner B. 1983. Reassessment of Ellman's reagent. Methods Enzymol 91:49-60.

Risau W. 1997. Mechanisms of angiogenesis. Nature 386:671-674.

Stathakis P, Fitzgerald M, Matthias LJ, Chesterman CN, Hogg PJ. 1997. Generation of angiostatin by reduction and proteolysis of plasmin: catalysis by a plasmin reductase secreted by cultured cells. J Biol Chem 272: 20641-20645.

Stocken LA, Thompson RHS. 1946. British Anti-Lewisite 2. Dithiol compounds as antidotes for arsenic. Biochem J 40:535-548.

Wall RT, Marker LA, Quadracci LJ, Striker GE. 1978. Factors influencing endothelial cell proliferation in vitro. J Cell Physiol 96: 203-213.

Claims

1. A method of treatment and/or prophylaxis of arthritis in a vertebrate comprising administering to said vertebrate in need of said treatment and/or prophylaxis a therapeutically effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt thereof, optionally together with a pharmaceutically acceptable carrier, diluent or excipient, wherein said compound of formula I is defined as

$$A\text{-}(L\text{-}Y)_p \qquad (I)$$

wherein

A comprises at least one substantially cell-membrane impermeable pendant group;

L comprises any suitable linker and/or spacer group;

Y comprises at least one arsenoxide or arsenoxide equivalent;

p is an integer from 1 to 10; and

the sum total of carbon atoms in A and L together, is greater than 6.

2. The method according to claim 1, wherein the compound is hydrophilic.

3. The method according to claim 1, wherein the compound is charged

4. The method according to claim 1, wherein the compound is uncharged

5. The method according to claim 1, wherein the compound is neutral.

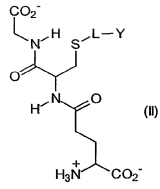
6. The method according to any one of claims 1 to 5, wherein A is selected from the group consisting of natural, unnatural and synthetic amino acids, hydrophilic amines, peptides, polypeptides, oligosaccharides, detectable groups, and thiol containing proteins, or a combination thereof.

7. The method according to claim 6 wherein the detectable group is biotin or a fluorophore.

8. The method of claim 7 wherein the fluorophore is selected from the group consisting of fluorescein and cy™5.5.

9. The method according to any one of claims 1 to 6, wherein A is selected from the group consisting of glutathione, glucosamine, cysteinylglycine, cysteic acid, aspartic acid, glutamic acid, lysine, and arginine, and wherein the sulfur atom of each sulfur containing compound may be optionally oxidised to form a sulfoxide or sulfone.

10. The method according to any one of claims 1 to 9, wherein A is glutathione, and wherein the compound is represented by Formula II:



(II)

wherein L comprises any suitable linker and/or spacer group, and Y comprises an arsenoxide or an arsenoxide equivalent.

11. The method according to any one of claims 1 to 10, wherein p is an integer from 1 to 5.

12. The method according to claim 11, wherein p is 1.

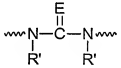
13. The method according to any one of claims 1 to 12, wherein L corresponds to $(XBX')_nB'$, and wherein:

n is an integer from 0 to 20,

X is selected from the group consisting of: NR-, S(O)-, -S(O)O-, -$S(O)_2$-, -$S(O)_2O$-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R_1)-, -P(O)(R_1)O-, or is absent;

B is selected from C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene or C_2-C_{10} acyl;

X' is selected from NR-, –O-, -S-, -Se-, -S-S-, S(O)-, -OS(O)-, OS(O)O-, -$OS(O)_2$, -$OS(O)_2O$-, -S(O)O-, -$S(O)_2$-, -$S(O)_2O$-, -OP(O)(R_1)-, -OP(O)(R_1)O-, -OP(O)(R_1)OP(O)(R_1)O-, -C(O)-, -C(S)-, -C(O)O-, C(S)O-, -C(S)S-, -P(O)(R_1)-, -P(O)(R_1)O-,



or is absent; wherein E is O, S, Se, NR or $N(R)_2^+$;

and

B' is C_1-C_{10} alkylene, C_2-C_{10} alkenylene, C_2-C_{10} alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_3-C_{10} heterocycloalkylene, C_5-C_{10} heterocycloalkenylene, C_6-C_{12} arylene, heteroarylene or is absent; and wherein

each R is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, OR_2 or C_2-C_{10} acyl;

R' is the same as R or two R' may be taken together with the nitrogen atoms to which they are attached to form a 5 or 6-membered saturated or unsaturated heterocyclic ring;

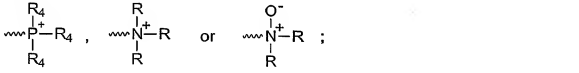
each R_1 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, OR_2 or NO_2;

each R_2 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl or $-C(O)R_5$;

each R_5 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, OH, SH or NO_2;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para, meta or ortho relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, heterocycloalkylene, heterocycloalkenylene, arylene, heteroarylene and acyl may be independently substituted with hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-OS(O)R_3$, $-S(O)_2R_3$, $-OS(O)_2R_3$, $-P(O)R_4R_4$, $-OP(O)R_4R_4$, $-N(R")_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$, N®2 or $-C(O)R_5$;

each R_3 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio or NO_2;

each R_4 is independently selected from hydrogen, C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkoxy, C_3-C_{10} alkenyloxy, C_3-C_{10} alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_3-C_{10} heterocycloalkyloxy, C_5-C_{10} heterocycloalkenyloxy, C_6-C_{12} aryloxy, heteroaryloxy, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, halo or NO_2;

R_6 is selected from C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_3-C_{10} heterocycloalkyl, C_5-C_{10} heterocycloalkenyl, C_6-C_{12} aryl, heteroaryl, C_1-C_{10} alkylthio, C_3-C_{10} alkenylthio, C_3-C_{10} alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_3-C_{10} heterocycloalkylthio, C_5-C_{10} heterocycloalkenylthio, C_6-C_{12} arylthio, heteroarylthio, $-S(O)R_3$, $-S(O)_2R_3$ or $-C(O)R_5$,

R" is the same as R or two R" taken together with the N atom to which they are attached may form a saturated, unsaturated or aromatic heterocyclic ring system;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

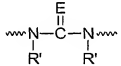
m is 1 to 5.

14. The method according to claim 13, wherein

X is selected from the group consisting of –C(O)-, -C(S)-, -C(O)O-, -C(S)O-, -C(S)S-, or is absent;

B is selected from C_1-C_5 alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_6-C_{12} arylene or C_2-C_5 acyl;

X' is selected from –O-, -S-, -NR-, -S-S-, -S(O)-, $-S(O)_2$-, $-P(O)(R_1)$-, $-OP(O)(R_1)$-, $OP(O)(R_1)O$-, $-OP(O)(R_1)OP(O)(R_1)O$-, –C(O)-, -C(S)-, -C(O)O-, -C(S)O-, -C(S)S-, -Se-,



or is absent; wherein E is O, S or $N(R)_2$+;

n is 0, 1 or 2; and

B' is C_1-C_5 alkylene, C_2-C_5 alkenylene, C_2-C_5 alkynylene, C_3-C_{10} cycloalkylene, C_5-C_{10} cycloalkenylene, C_6-C_{12} arylene or is absent; and wherein

each R is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, OR_2 or C_2-C_{10} acyl;

R' is the same as R;

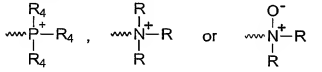
each R_1 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, OR_2 or $N(R)_2$;

each R_2 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl or -C(O)R_5;

each R_5 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_3-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH or N(R)$_2$;

wherein each instance of arylene may have substituents A and X or X and Y in a para, meta or ortho relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -S(O)$_2R_3$, -OS(O)$_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -N(R")$_2$, NRC(O)(CH$_2$)$_m$Q, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, -S(O)R_3, -S(O)$_2R_3$, -P(O)$(R_4)_2$, N(R)$_2$ or -C(O)R_5;

each R_3 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio or N(R)$_2$;

each R_4 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_5 alkynyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_5 cycloalkylthio, C_5-C_5 cycloalkenylthio, C_6-C_{12} arylthio, halo or N(R)$_2$;

R_6 is independently selected from C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_5 alkynylthio, C_3-C_{10} cycloalkylthio, C_5-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, -S(O)R_3, -S(O)$_2R_3$ or –C(O)R_5,

R" is the same as R;

Q is selected from halogen and –OS(O)$_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

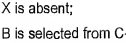
m is 1 to 5.

15. The method according to claim 13, wherein

X is absent;

B is selected from C_1-C_5 alkylene, C_6-C_{12} arylene or C_2-C_5 acyl;

X' is selected from –O-, -S-, -NR-, -S-S-, -S(O)-, -$S(O)_2$-, -P(O)(R_1)-, –C(O)-, -C(S)-, -C(O)O-, -C(S)O-, -Se-,



or absent; wherein E is O, S or $N(R)_2^+$;

n is 0, 1 or 2; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and wherein

each R is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, OR_2 or C_2-C_5 acyl;

R' is the same as R;

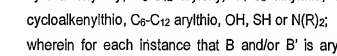
each R_1 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, halo, OR_2 or N(R)2;

each R_2 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl or -C(O)R_5;

each R_5 is independently selected from hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_5 alkenyloxy, C_3-C_{10} cycloalkyloxy, C_5-C_{10} cycloalkenyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_5 alkenylthio, C_3-C_{10} cycloalkylthio, C_6-C_{10} cycloalkenylthio, C_6-C_{12} arylthio, OH, SH or N(R)2;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para, meta or ortho relationship, and

wherein each alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkenylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, -S(O)R_3, -OS(O)R_3, -$S(O)_2R_3$, -$OS(O)_2R_3$, -P(O)R_4R_4, -OP(O)R_4R_4, -N(R")2, -NRC(O)$(CH_2)_mQ$, -C(O)R_5,



wherein R, R_1 and R_5 are as defined above; and

R_{2a} is selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, -S(O)R_3, -$S(O)_2R_3$, -P(O)$(R_4)_2$ and –C(O)R_5;

each R_3 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio or $N(R)_2$;

each R_4 is independently selected from hydrogen, C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_3-C_{10} cycloalkyloxy, C_6-C_{12} aryloxy, halo or $N(R)_2$;

R_6 is selected from C_1-C_5 alkyl, C_3-C_{10} cycloalkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_3-C_{10} cycloalkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ or $-C(O)R_5$,

R" is the same as R;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

16. The method according to claim 13, wherein

X is absent;

B is selected from C_1-C_5 alkylene, C_6-C_{12} arylene or C_2-C_5 acyl;

X' is selected from –O-, -S-, -NR-, -C(O)-, -C(O)O-, or is absent;

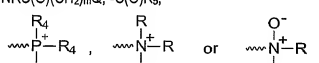
n is 1; and

B' is C_1-C_5 alkylene, C_6-C_{12} arylene or is absent; and

R is selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl or C_2-C_5 acyl;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalents), may be in a para, meta or ortho relationship, and

wherein each alkylene, arylene, and acyl may be independently substituted with hydrogen, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl, C_3-C_{10} cycloalkyl, C_5-C_{10} cycloalkenyl, C_6-C_{12} aryl, halo, cyano, cyanate, isocyanate, OR_{2a}, SR_6, nitro, arsenoxide, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)R_4R_4$, $-N(R")_2$, $-NRC(O)(CH_2)_mQ$, $-C(O)R_5$,



wherein each R is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl or C_2-C_5 acyl;

R_{2a} is selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, $-S(O)R_3$, $-S(O)_2R_3$, $-P(O)(R_4)_2$ or $-C(O)R_5$;

each R_3 is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, or C_6-C_{12} arylthio;

each R_4 is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, halo or $N(R)_2$;

each R_5 is independently selected from hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkoxy, C_6-C_{12} aryloxy, C_1-C_5 alkylthio, C_6-C_{12} arylthio, OH, SH or $N(R)_2$;

R_6 is selected from C_1-C_5 alkyl, C_6-C_{12} aryl, C_1-C_5 alkylthio, C_6-C_{12} arylthio, $-S(O)R_3$, $-S(O)_2R_3$ or $-C(O)R_5$,

R" is the same as R above;

Q is selected from halogen and $-OS(O)_2Q_1$; wherein Q_1 is selected from C_1-C_4 alkyl, C_1-C_4 perfluoroalkyl, phenyl, *p*-methylphenyl; and

m is 1 to 5.

17. The method according to claim 13, wherein

X is absent;

B is C_2-C_5 acyl;

X' is NR;

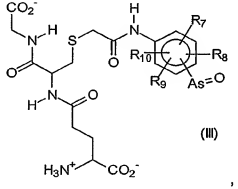
n is 1;

B' is phenylene; and

R is H;

wherein for each instance that B and/or B' is arylene, the substituents directly attached to the respective arylene rings (including arsenoxide or arsenoxide equivalent), may be in a para-, meta- or ortho- relationship.,

18. The method according to any one of claims 1-17 represented by Formula III:



(III)

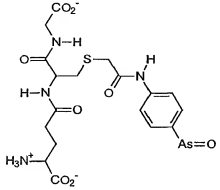
, and wherein

R_7 to R_{10} are independently selected from the group consisting of: hydrogen, C_1-C_5 alkyl, C_6-C_{12} aryl, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, $-OS(O)_2R_3$ or $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ or $-OS(O)_2$-*p* tolyl.

19. The method according to claim 18, wherein R_7 to R_{10} are independently selected from the group consisting of: hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, methyl, ethyl, iso-propyl, tert-butyl, phenyl and $-NHC(O)CH_2Q$ wherein Q is halogen, $-OS(O)_2CH_3$, $-OS(O)_2C_6H_5$ or $-OS(O)_2$-*p* tolyl.

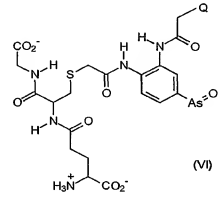
20. The method according to claim 18 or 19, wherein the arsenoxide (-As=O) group is at the 4-position of the phenylene ring.

21. The method according to any one of claims 1 to 17 wherein the compound is 4-(*N*-(*S*-glutathionylacetyl)amino)phenylarsenoxide (GSAO) and is represented by Formula V:



(V)

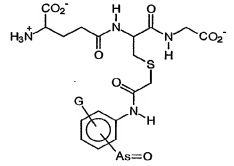
22. The method according to any one of claims 1 to 17 wherein the compound is represented by Formula VI:



(VI)

wherein Q is any halogen.

23. The method according to any one of claims 1 to 17 wherein the compound is represented by Formula VII:



(VII)

wherein G is selected from the group consisting of: hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, C_1-C_5 alkyl and C_6-C_{12} aryl and -NHC(O)CH_2Q wherein Q is halogen, -$OS(O)_2CH_3$, -$OS(O)_2C_6H_5$ or –$OS(O)_2$-*p* tolyl.

24. The method according to claim 23, wherein G is selected from the group consisting of: hydrogen, halogen, hydroxy, amino, nitro, carboxy, C_1-C_5 alkoxy, methyl, ethyl, iso-propyl, tert-

butyl, phenyl, and -NHC(O)CH_2Q wherein Q is halogen, -$OS(O)_2CH_3$, -$OS(O)_2C_6H_5$ or –$OS(O)_2$-*p* tolyl.

25. The method according to claim 23 or 24, wherein G is selected from the group consisting of hydroxy, fluorine, amino, and nitro.

26. The method according to any one of claims 23 to 25 wherein the activity of the arsenic atom may be modified by the group G, when G and the arsenic atom are in an ortho- or para-relationship to one another.

27. The method according to any one of claims 1 to 26, wherein the arsenoxide group (-As=O) is replaced by an arsenoxide equivalent.

28. The method according to claim 27, wherein said arsenoxide equivalent is in the form of -$D(Z_1)(Z_2)$, wherein D is As Sn, Sb, Ge, and Z_1 and Z_2 are labile groups, and wherein Z_1 and Z_2, may be identical or different, and may either be connected or independent from each other (bound only to the arsenic atom).

29. The method according to claim 28, wherein wherein Z_1 and Z_2 are selected from OH, C_1-C_{10} alkoxy, C_6-C_{10} aryloxy, C_1-C_{10} alkylthio, C_6-C_{10} arylthio, C_1-C_{10} alkylseleno, C_6-C_{10} arylseleno, OH, OR, SR, SeR, F, Cl, Br and I, and wherein R is an alkyl or an aryl group.

30. The method according to any one of claims 1 to 29, wherein said compound is linked to a detector group.

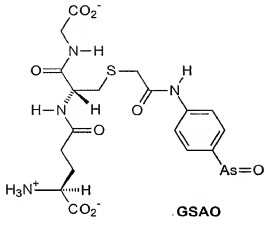
31. The method of claim 30 wherein said detector group is selected from the group consisting of fluorophore, biotin, radionucleotide, biotin, fluorescein, and a group comprising a transition element.

32. The method according to claim 30 or 31 wherein the detector group is biotin.

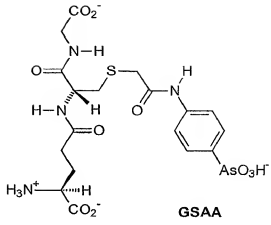
33. The method according to claim 31 wherein the radionucleotide is selected from the group consisting of ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S, ^{125}I, ^{131}I, ^{123}I, ^{111}In, ^{105}Rh, ^{153}Sm, ^{67}Cu, ^{67}Ga, ^{166}Ho, ^{177}Lu, ^{186}Re, ^{188}Re, and ^{99m}Tc.

34. The method according to claim 33, wherein the radionucleotide is selected from the group consisting of ^{3}H or ^{14}C.

35. The method of any one of claims 1-34, wherein the arthritis is selected from the group consisting of calcific periarthritis, enteropathic arthritis, chronic arthritis, gout, hand osteoarthritis, hip arthritis, knee osteoarthritis, thumb arthritis, Jaccoud's arthritis, juvenile osteoarthritis, oligoarthritis, polyarthritis, peripheral arthritis, psoriatic arthritis, rheumatoid arthritis and septic arthritis.



GSAO



GSAA

FIGURE 1

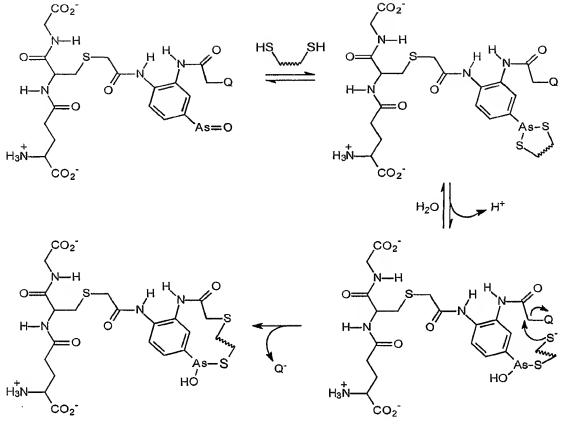


FIGURE 2

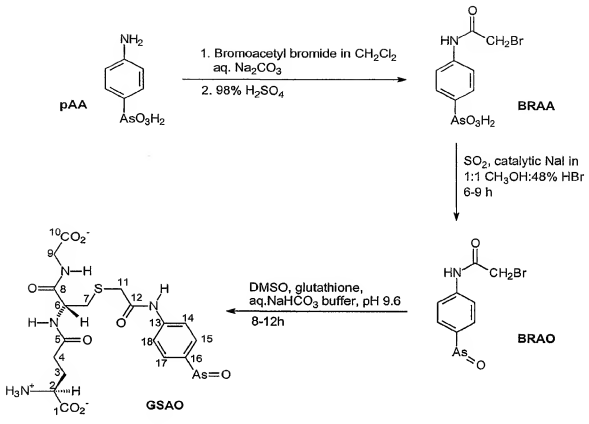

pAA
1. Bromoacetyl bromide in CH_2Cl_2
aq. Na_2CO_3
2. 98% H_2SO_4
BRAA
SO_2, catalytic NaI in
1:1 CH_3OH:48% HBr
6-9 h
BRAO
DMSO, glutathione,
aq.$NaHCO_3$ buffer, pH 9.6
8-12h
GSAO

FIGURE 3

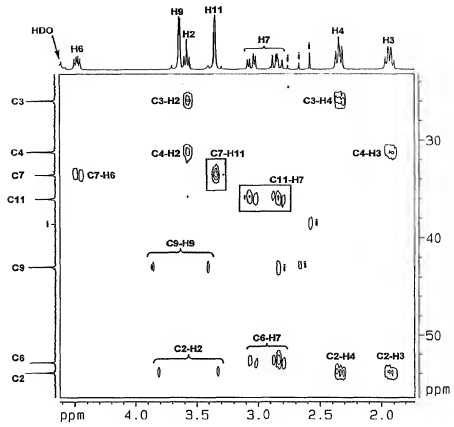
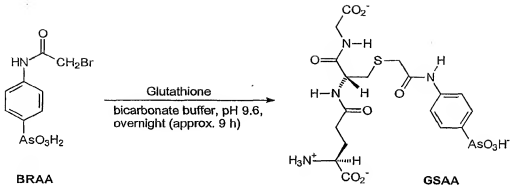

HDO
H6
H9
H2
H11
H7
H4
H3
C3
C4
C7
C11
C9
C6
C2
C3-H2
C3-H4
C4-H2
C7-H11
C4-H3
C7-H6
C11-H7
C9-H9
C2-H2
C6-H7
C2-H4
C2-H3
30
40
50
ppm
ppm
4.0
3.5
3.0
2.5
2.0

FIGURE 4



BRAA GSAA

FIGURE 5

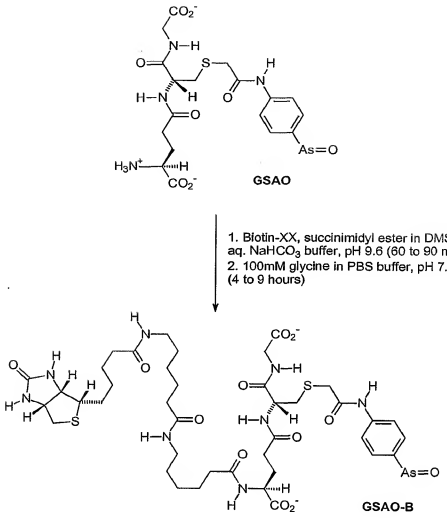
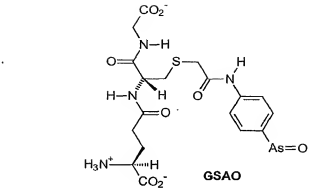
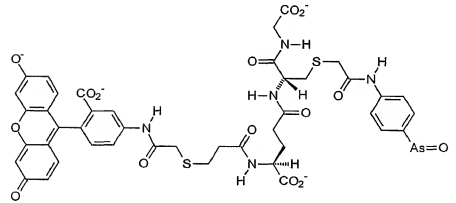

GSAO
1. Biotin-XX, succinimidyl ester in DMSO, aq. NaHCO3 buffer, pH 9.6 (60 to 90 mins)
2. 100mM glycine in PBS buffer, pH 7.3 (4 to 9 hours)
GSAO-B

FIGURE 6



GSAO

1. Fluorescein-EX, succinimidyl ester in DMSO, aq. $NaHCO_3$ buffer, pH 9.6 (60 to 90 mins);
2. 100mM glycine in PBS buffer, pH 7.3 (4 to 9 hours)



GSAO-F

FIGURE 7

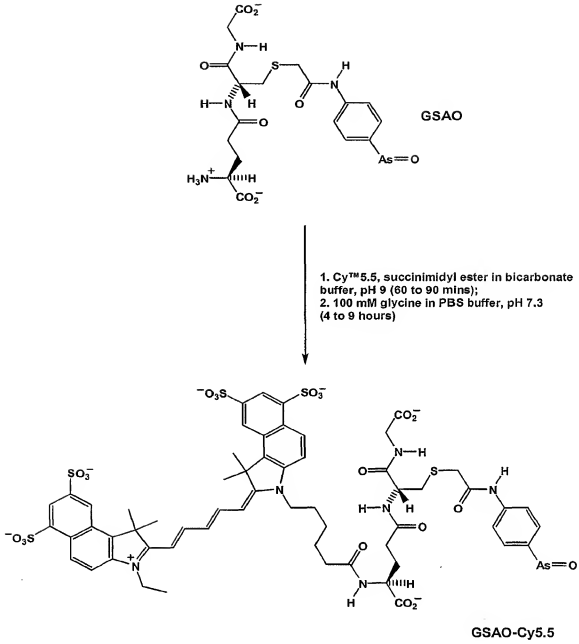

GSAO
1. Cy™5.5, succinimidyl ester in bicarbonate buffer, pH 9 (60 to 90 mins);
2. 100 mM glycine in PBS buffer, pH 7.3 (4 to 9 hours)
GSAO-Cy5.5

FIGURE 8

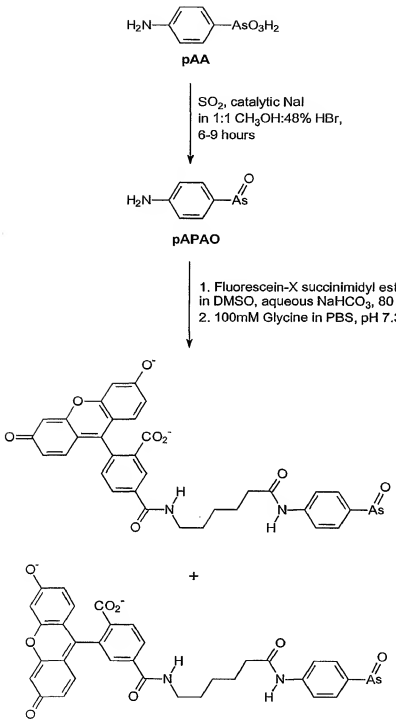

H2N
AsO3H2
pAA
SO2, catalytic NaI
in 1:1 CH3OH:48% HBr,
6-9 hours
H2N
As
O
pAPAO
1. Fluorescein-X succinimidyl ester (mixture of isomers)
in DMSO, aqueous NaHCO3, 80 mins
2. 100mM Glycine in PBS, pH 7.3, 4-9 hours
O-
O
CO2-
O
H
N
O
N
H
O
As
+
FXAO, mixture of isomers

FIGURE 9

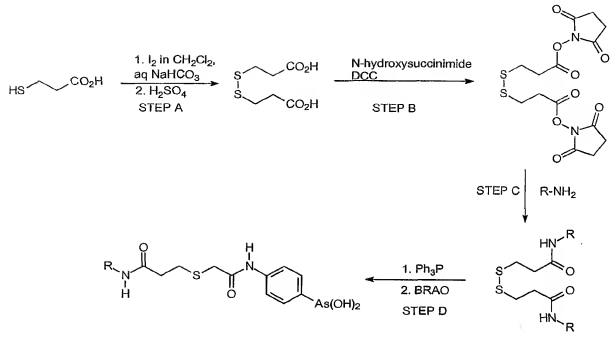

1. I_2 in CH_2Cl_2,
aq $NaHCO_3$
2. H_2SO_4
STEP A
N-hydroxysuccinimide
DCC
STEP B
STEP C
R-NH_2
1. Ph_3P
2. BRAO
STEP D
As(OH)$_2$

FIGURE 10

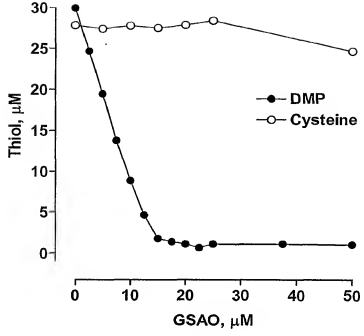


FIGURE 11

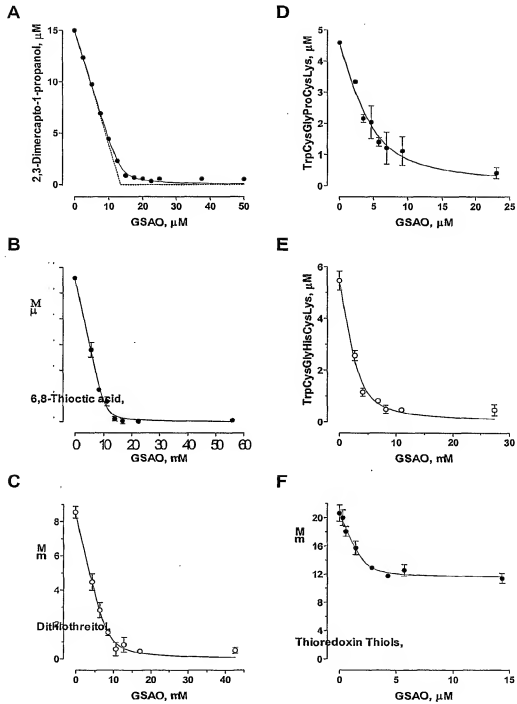

A
2,3-Dimercapto-1-propanol, µM
GSAO, µM
D
TrpCysGlyProCysLys, µM
GSAO, µM
B
6,8-Thioctic acid,
GSAO, mM
E
TrpCysGlyHisCysLys, µM
GSAO, mM
C
Dithiothreitol,
GSAO, mM
F
Thioredoxin Thiols,
GSAO, µM

FIGURE 12

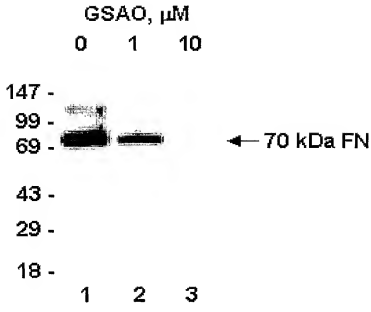


FIGURE 13

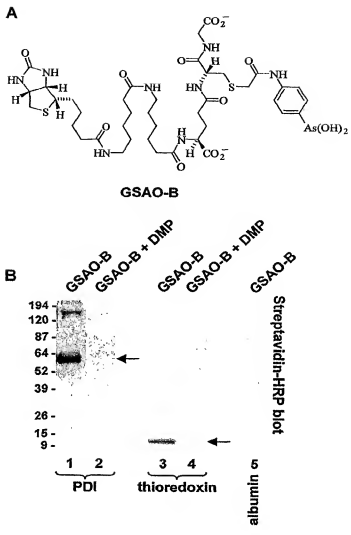

A
GSAO-B
B
GSAO-B
GSAO-B + DMP
GSAO-B
GSAO-B + DMP
GSAO-B
Streptavidin-HRP blot
194
120
87
64
52
39
26
15
9
1
2
3
4
5
PDI
thioredoxin
albumin

FIGURE 14

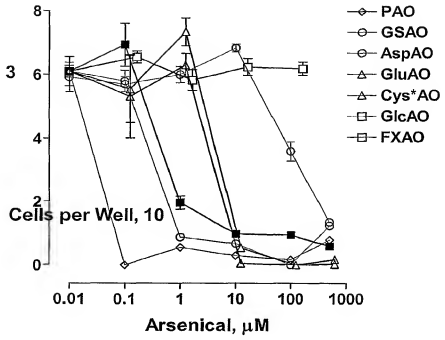

PAO
GSAO
AspAO
GluAO
Cys*AO
GlcAO
FXAO
Cells per Well, 10
3
8
6
4
2
0
0.01
0.1
1
10
100
1000
Arsenical, µM

FIGURE 15

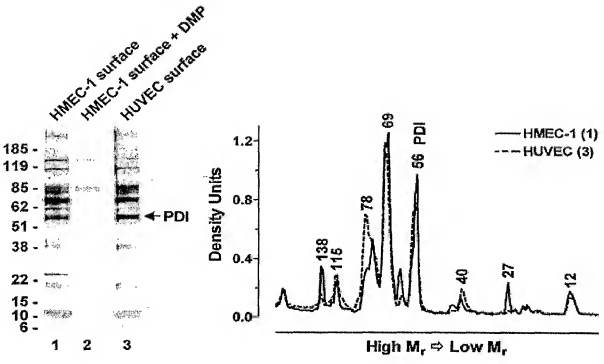

HMEC-1 surface
HMEC-1 surface + DMP
HUVEC surface
185 -
119 -
85 -
62 -
51 -
38 -
22 -
15 -
10 -
6 -
PDI
1
2
3
Density Units
1.2
0.8
0.4
0.0
138
116
78
69
56 PDI
40
27
12
HMEC-1 (1)
HUVEC (3)
High M$_r$ ⇨ Low M$_r$

FIGURE 16

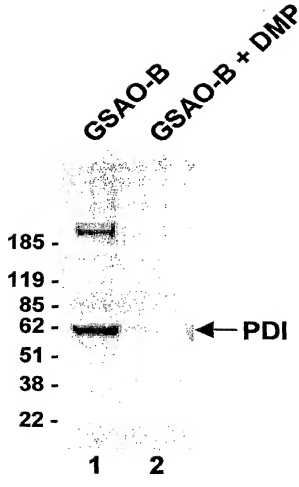


FIGURE 17

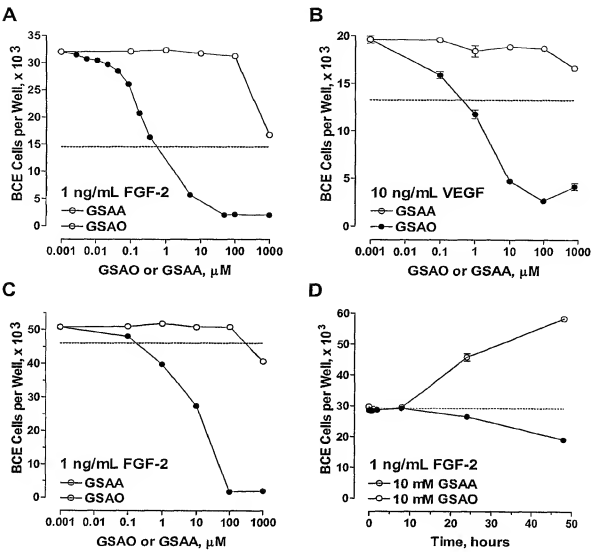

A
BCE Cells per Well, x 10^3
1 ng/mL FGF-2
GSAA
GSAO
GSAO or GSAA, μM
B
BCE Cells per Well, x 10^3
10 ng/mL VEGF
GSAA
GSAO
GSAO or GSAA, μM
C
BCE Cells per Well, x 10^3
1 ng/mL FGF-2
GSAA
GSAO
GSAO or GSAA, μM
D
BCE Cells per Well, x 10^3
1 ng/mL FGF-2
10 mM GSAA
10 mM GSAO
Time, hours

FIGURE 18

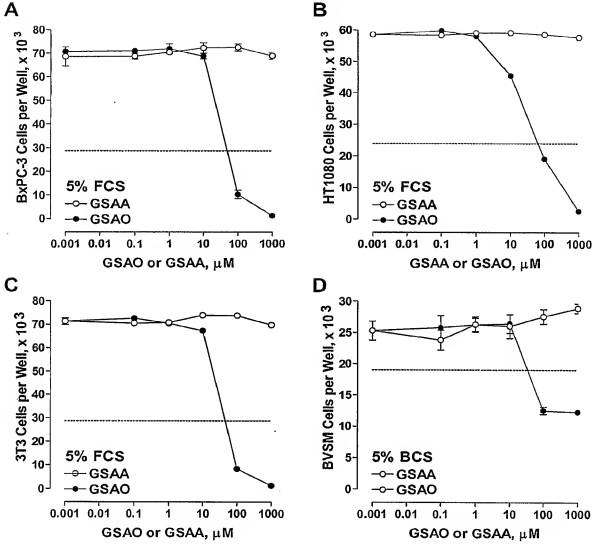

A
BxPC-3 Cells per Well, x 10 3
5% FCS
GSAA
GSAO
GSAO or GSAA, µM
B
HT1080 Cells per Well, x 10 3
5% FCS
GSAA
GSAO
GSAA or GSAO, µM
C
3T3 Cells per Well, x 10 3
5% FCS
GSAA
GSAO
GSAO or GSAA, µM
D
BVSM Cells per Well, x 10 3
5% BCS
GSAA
GSAO
GSAO or GSAA, µM

FIGURE 19

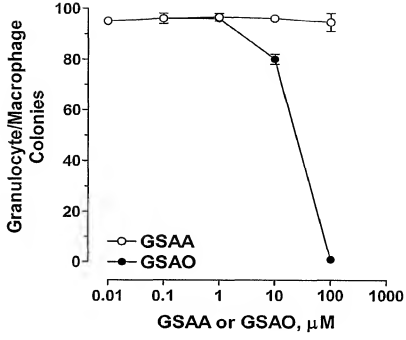

Granulocyte/Macrophage Colonies
100
80
60
40
20
0
GSAA
GSAO
0.01
0.1
1
10
100
1000
GSAA or GSAO, μM

FIGURE 20

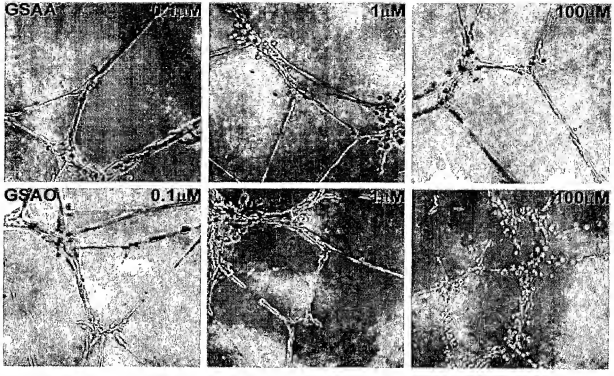


FIGURE 21

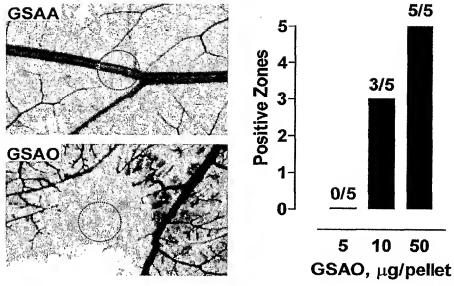

GSAA
GSAO
Positive Zones
5
4
3
2
1
0
0/5
3/5
5/5
5
10
50
GSAO, μg/pellet

FIGURE 22

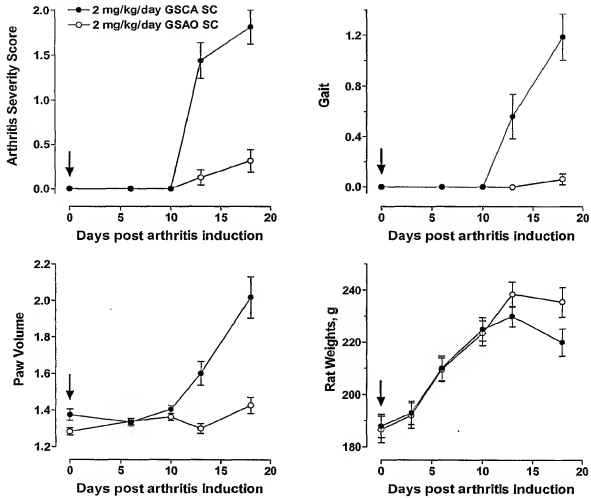

2 mg/kg/day GSCA SC
2 mg/kg/day GSAO SC
Arthritis Severity Score
Days post arthritis induction
Gait
Days post arthritis induction
Paw Volume
Days post arthritis induction
Rat Weights, g
Days post arthritis induction

Figure 23

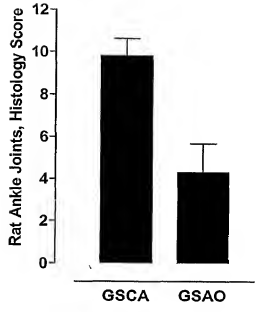


Figure 24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/00310

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61K 31/285, A61P 19/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU:IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPAT: aresenoxide, arsenic, arsenical, arsen, arthritis

MEDLINE: arsenoxide, arsenic, arsenical, arthritis.

CA: trypanothione, arsenic.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Fairlamb et al, "Trypanothione is the primary target for arsenical drugs against African trypanosomes" Proc. Natl. Acad. Sci (1989) 86, 2607-2611. See Fig 2 and pages 2607-2608.	1-35
A	Fairlamb et al, "Metabolism and functions of trypanothione in the kinetoplastida" Ann Rev. Microbiol (1992) 46. 695-729, See 707-708.	1-35

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" Earlier application or patent but published on or after the international filing date

"L" Document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" Document referring to an oral disclosure, use, exhibition or other means

"P" Document published prior to the international filing date but later than the priority date claimed

"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" Document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" Document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" Document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 May 2002	29 MAY 2002
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**SHUBHRA CHANDRA** Telephone No : (02) 6283 2264

C (Continuation). **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Cunningham et al, Mechanism of inhibition of trypanothione reductase and glutathione reductase by trivalent organic arsenicals" Eur. J. Biochem (1994) 221, 285-295. See table 1 p 288	1-35
P, X	WO 01/21628 A1 (UNISEARCH LIMITED) 29 March 2001, Whole Specification.	1-35

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU02/00310

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	200121628	AU	200076320

END OF ANNEX